

LUMINESCENCE STUDIES IN ORGANIZED MEDIA

BY

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This dissertation is dedicated to the memory of my dear father, Walter Timothy Murdoch O'Reilly and to my cousin Catherine (Aunt Kate) Pigott, both of whom did not live to see the completion of this project. It is also dedicated to my dear mother, Christine O'Reilly, and to my sisters, Edith, Avis, and Marion, and to my brother, Walter. It is also dedicated to those of my relatives and friends who provided financial and emotional support throughout my time in graduate school.

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Abstract of Dissertation Presented to the Graduate School
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LUMINESCENCE STUDIES IN ORGANIZED MEDIA

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Various types of organized media are used to alter and/or enhance the luminescence properties of several molecules on solid substrate and in fluid solution. The effect of surfactants of different ionic character on the paper substrate room temperature phosphorescence (PS-RTP) of several polyaromatic hydrocarbons and carbazole was studied. Thallium (I) nitrate was used as a heavy atom perturber. When the analytes were spotted from anionic micellar solutions, enhancement factors of the sensitivity ranging between 2 and 9 were obtained. By contrast, the PS-RTP signal was totally quenched in the presence of the cationic surfactant. Absolute limits of detection in the range of 0.2-3 ng were obtained in the presence of sodium dodecylsulfate. A discussion on the possible mechanisms producing the observed effects is included.

The effects of surfactants of various ionic character and thallium (I) nitrate on the PS-RTP of eleven phenothiazine derivatives are described. Enhancement factors within the range 1.5-10 are observed after the addition of sodium dodecylsulfate and dodecyltrimethylammonium

chloride and thallium (I) nitrate. The influence of surfactant on phosphor characteristics as well as effects of moisture are discussed. Limits of detection (LODs) were in the range 0.2-2 ng.

Luminescence of organic molecules is observed in DHP vesicles in the absence and presence of magnetite encapsulated in the vesicles. Room temperature phosphorescence (RTP) calibration curves for carbazole were constructed at 297/440 nm in the absence and presence of magnetite. The linear dynamic ranges (LDRs) were found to be over 2.5 orders of magnitude. The LODs were found to be 30 ng/ml and 9 ng/ml in the absence and presence of magnetite, respectively. The effect of the internal magnetic field on the luminescence properties of various molecules is discussed.

The effect of brominated alcohols and their nonbrominated counterparts on the luminescence properties of various molecules in cyclodextrins is investigated. The observed results may be explained by the stabilities of the ternary complex formed. Analytical figures of merit for various molecules are given. LDRs were about four orders of magnitude; LODs were in the nanomolar range.

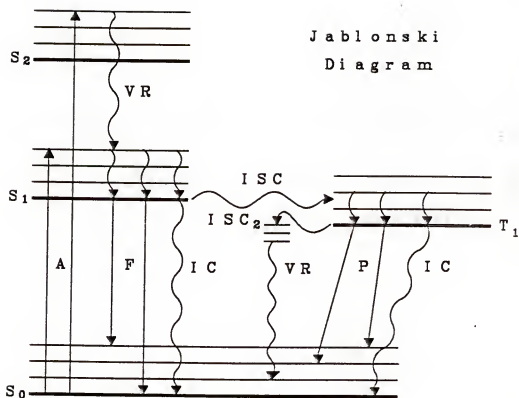
CHAPTER 1
THEORETICAL AND PRACTICAL ASPECTS OF LUMINESCENCE
IN ORGANIZED MEDIA AND ON SOLID SUBSTRATE

Introduction

Molecular Luminescence Spectroscopy (MLS) has emerged to become one of the most useful tools in experimental biology and chemistry. This is particularly important in fields such as the biomedical sciences where low concentrations of drugs, metabolites, and toxins must routinely be monitored, or in the environment where minute amounts of potentially carcinogenic and mutagenic substances such as polyaromatic hydrocarbons (PAHs) exist and must be able to be detected.

Organized media, such as aqueous micelles, cyclodextrins, and vesicles have been found to greatly increase selectivities and sensitivities of many molecules in molecular luminescence studies compared to those obtained in homogeneous solution studies. The use of organized media also has provided an opportunity to observe delayed fluorescence and phosphorescence in fluid solution which ordinarily cannot be observed in homogeneous solutions at room temperature. The observation of these phenomena can be attributed to the stabilization of the emitting species which are organized on a molecular basis in these systems. Molecular luminescence in organized media is also enhanced by the effective shielding from quenchers in solution and by the higher efficiency of spin-orbital coupling processes with the heavy atom in these systems.

Fig. 1-1. Jablonski Diagram showing the various radiative and nonradiative transitions in a molecule undergoing absorption (A) from the ground electronic state (S_0) to any of the various vibrational levels of the electronically excited states (S_1 and S_2). (VR, vibrational relaxation; IC, internal conversion; F, fluorescence; ISC, intersystem crossing to the lowest triplet state (T_1); ISC₂, reverse intersystem crossing; P, phosphorescence.



The analytical measurements of the fluorescence or phosphorescence of components adsorbed on solid materials constitute the general area of solid-surface luminescence analysis. A considerable number of solid materials have been used in chemical analysis. These include filter paper, silica gel, aluminum oxide, silicone rubber, sodium acetate, potassium bromide, and cellulose.

It has been found that only certain substrates are useful for inducing room temperature phosphorescence (RTP) from adsorbed organic compounds. Cellulose filter paper is the most widely used solid surface to date for inducing RTP. However, several aspects of solid surface RTP, such as binding mechanisms and interactions of the analyte with the substrate, are not yet fully understood.

Photophysical Processes in Isolated Molecules

At equilibrium, a molecule has a thermal distribution at the lowest vibrational and rotational levels of the ground state S_0 . When a molecule absorbs excitation energy, it is elevated from S_0 to some vibrational level of one of the excited singlet states S_n in the manifold, $S_1, \dots, S_n$ (1). Although the absorption process is extremely rapid, taking about 10^{-15} s, the sequence of events that returns the excited molecule to its ground state is considerably slower, taking from 10^{-14} s to several seconds (2). The main photophysical processes involved in the deactivation of the molecule in its excited state include vibrational relaxation (VR), internal conversion (IC), fluorescence (F), intersystem crossing (ISC), and phosphorescence (P) (1). These processes are illustrated in the Jablonski diagram (Figure 1-1).

Vibrational Relaxation (VR). Upon arriving in the electronically excited state, the excited molecule may be in a vibrationally excited

state (with $v > 0$). The molecule will then begin to vibrate with a frequency characteristic of the vibrationally excited state, giving up stepwise its excess vibrational energy in the form of infrared quanta or in the form of kinetic energy imparted to other molecules with which it collides. Within the lifetime of a few vibrations (10^{-14} - 10^{-12} s), it will have thermally relaxed to the lowest vibrational level of the electronically excited singlet state (i.e., the electronically excited molecule attains thermal equilibrium with the environment (2)).

Internal Conversion (IC). From the S_n state, the molecule deactivates to the isoenergetic vibrational level of a lower electronic state such as S_{n-1} , via an IC process. The IC processes are transitions between states of the same multiplicity. The molecule subsequently deactivates to the lowest vibronic levels of S_{n-1} via a VR process. By a succession of IC processes immediately followed by VR processes, the molecule deactivates rapidly to S_1 . As a result, a molecule may be excited to S_1 or higher excited states, S_n , depending on the excitation energy being used, but the emission takes place only from the lowest excited electronic state, S_1 (1). The internal conversion process is very rapid taking about 10^{-12} s. The average lifetime of the thermally equilibrated, lowest excited singlet state, however, is of the order of 10^{-8} s. Consequently, even if a molecule cannot pass efficiently from its lowest excited singlet state to the ground state by internal conversion, it may undergo other processes during the lifetime of the lowest excited single state which may compete with fluorescence (2).

Fluorescence (F). From the S_1 state, the molecule may either further deactivate to S_0 in a radiationless fashion via IC and VR processes or emit a photon without a change in spin multiplicity. The latter $S_1 \rightarrow S_0$

transition is known as fluorescence. The energy of the photon corresponds to the difference in energy between the lowest vibrational level of the excited state and the vibrational level of the ground state. The fluorescence spectrum primarily provides information about the vibrational structure of the ground state. The lifetimes of the fluorescent process are typically of the order of 10^{-8} s (1,2).

Intersystem Crossing (ISC). From the S_1 state, the molecule may undergo a transition to some vibrational level of the triplet manifold via a mechanism known as an intersystem crossing (ISC) process. The ISC process involves a change in spin multiplicity. This change in multiplicity involves a change in spin angular momentum, a process which is classically forbidden (i.e., has zero probability). However, spin-forbidden transitions can occur under certain conditions, but the probability is much lower than for the corresponding process with no change of spin [10^{-3} - 10^{-6} times than that of allowed transitions (3,4)]. The result of the much lower probability processes is that the mean lifetime of the spin-forbidden process is much longer than the corresponding spin-allowed process. Spin-allowed vibrational transitions (VR and IC) have mean lifetimes of $\sim 10^{-14}$ s. Therefore, the mean lifetime of the spin-forbidden vibrational transition (ISC) is about 10^{-8} s, which is about the mean lifetime of a fluorescing molecule (2).

Consequently, while ISC is too slow to compete with IC, it is of the proper time scale to compete with fluorescence for deactivation of the lowest excited singlet state. Moreover, while most aromatic molecules undergo some degree of ISC from the lowest-excited singlet state, those molecules containing atoms of high atomic number, n-electrons or transition metal ions employ intersystem crossing as an efficient, if not

exclusive, means of deactivating the lowest excited single state. This is the result of a phenomenon called spin-orbital coupling, which consists of the addition of the spin and orbital angular momenta, vectorially, so that the spin angular momentum of the molecule is not well defined. This partially removes the distinctiveness of the forbiddenness of singlet-triplet transitions (2). The molecule then relaxes to the lowest vibrational level of T_1 via successive IC and VR processes.

From the T_1 state, the molecule may return to the ground state S_0 , either by a radiationless deactivation path (ISC_2 , shown in Figure 1-1) or by the emission of a photon. The latter radiative transition is known as phosphorescence. The radiationless deactivation that quite often competes with phosphorescence is the reverse ISC process (ISC_2). This process, which is often dominant, determines the observed phosphorescence lifetime.

The nonradiative rate constant corresponding to the ISC process $T_1 \rightarrow S_0$ is strongly dependent on the magnitude of the energy gap $\Delta E (T_1 - S_0)$. The rate constant decreases exponentially with increasing ΔE (1).

Phosphorescence (P). The radiative transition from the lowest triplet state to the ground singlet state is longer lived than fluorescence because of the spin forbiddenness (low probability) of the former and is called phosphorescence. Phosphorescence is, like fluorescence, most likely to occur in molecules having restricted vibrational freedom and is thus most often observed in aromatic molecules and their derivatives. However, all aromatic molecules are not phosphorescent. Because triplet states are so long lived (10^{-5} s to several seconds), chemical and physical processes in solution as well as internal conversion compete effectively with phosphorescence for deactivation of the lowest excited triplet state. Except for the shortest

lived phosphorescence, collisional deactivation by solvent molecules, quenching by paramagnetic species, photochemical reactions, and energy transfer processes normally preclude the observation of phosphorescence in fluid media. Until quite recently under special conditions, phosphorescence was normally studied in the glassy state at liquid nitrogen temperature, in solution in very viscous liquids where collisional processes cannot completely deactivate the triplet state and in low pressure gases. Phosphorescence usually originates from the lowest vibrational level of the lowest triplet state and terminates in any of several vibrational levels of the ground state (2).

The quantum yield (quantum efficiency) of phosphorescence (ϕ_p) is defined by

$$\phi_p = \phi_{st} \cdot \frac{k_p}{k_p + \Sigma k_j} \quad (1-1)$$

where k_p is the molecular probability of phosphorescence, Σk_j is the sum of the rate constants of all unimolecular radiationless deactivation processes competing with phosphorescence for deactivation of the lowest triplet state, and ϕ_{st} is the efficiency of singlet-triplet intersystem crossing from the lowest excited singlet state. It should be noted that ϕ_p represents the fraction of the total number of molecules originally excited to the lowest excited singlet state that ultimately phosphoresces and is thus a three state quantum yield (2).

Delayed Fluorescence (DF). Occasionally, in rigid and viscous media, a second long lived emission band is observed in addition to the phosphorescence. The second band is of higher frequency (shorter wavelength) than the phosphorescence, and if the molecule also shows

fluorescence at the temperature of measurement, the frequency of the extraneous band coincides with the frequency of fluorescence, but its decay time is similar to that of the phosphorescence. This phenomenon, known as delayed fluorescence, is a result of the thermal excitation of a molecule in the lowest excited triplet state back to the lowest excited singlet state (i.e., reverse intersystem crossing) followed by fluorescence.

In rigid media, the thermal excitation is accomplished by warming the sample or by statistical fluctuations in the thermal energy of the triplet state. However, in viscous media where there is some mobility, the collision of two triplet molecules results in thermal excitation of one and the internal conversion of the other to the ground state.

Two types of delayed fluorescence may be distinguished, an E-type and a P-type. E-type delayed fluorescence is exhibited only by certain molecules which have a small energy gap between the lowest excited singlet and triplet states, making thermal repopulation of the lowest excited singlet state probable. P-type fluorescence may occur in molecules with substantial differences in energy between the lowest excited singlet and triplet states. The collision of two triplet molecules results in the thermal excitation of one and the internal conversion of the other to the ground state. This process is also known as triplet-triplet annihilation. Because two triplet molecules are required to produce one P-type delayed fluorescence event, fairly high concentrations of the absorbing species are usually required (2,3).

Lifetimes. The phosphorescence lifetime τ_p is defined as the time required for the emission to decrease to $1/e$ of its original intensity

following a δ -pulse excitation. If $I_p(t)$ is the phosphorescence intensity at time t and I_p^0 is the intensity at $t = 0$, then

$$I_p(t) = I_p^0 \exp\left(-\frac{t}{\tau_p}\right) \quad (1 - 2)$$

It is important to differentiate the intrinsic natural lifetime τ^* from the observed lifetime τ for a given radiative process. The intrinsic natural lifetime τ^* is measured only when there is no radiationless deactivation process competing with the emission, that is, when $\phi_p = 1.0$ for phosphorescence (1).

Due to quenching process, the natural lifetime τ_p^* of the triplet state cannot be determined directly by experiment but can be calculated by integrating the singlet-triplet absorption band (1)

$$\tau_p^* = \frac{1}{k_p} = \left(\frac{(\bar{\nu})^2}{3.47 \times 10^8} \cdot \frac{g_l}{g_u} \int \epsilon \, d\bar{\nu} \right)^{-1} \quad (1 - 3)$$

where $\bar{\nu}$ is the average frequency of the transition.

ϵ is the molar extinction coefficient

g_l and g_u are the degeneracies of the lower and upper states, respectively.

The relationship between τ_p^* , τ_p and ϕ_p is given by

$$\tau_p = \tau_p^* \phi_p \quad (1 - 4)$$

If one knows the value of one of the two parameters τ^* and ϕ_p , one can derive the value of the other by determining τ_p experimentally.

Heavy Atom Effect. The rate of ISC can be enhanced by the presence of atoms of high atomic number. These atoms of high atomic numbers are known as heavy atoms; the heavy atom may either be internal or external.

The internal heavy atom is directly bonded to the molecule, whereas the external heavy atom is added to the microenvironment. The addition of heavy atoms to the environment enhances the rate of ISC processes, increasing the quantum yield, and decreases the phosphorescence lifetime in the molecule (1,2).

The heavy atom added to the environment of the molecule causes the spin and orbital angular momenta of the solute to interact strongly with each other. As a result, the selection rule forbidding changes of spin angular momentum in electronic transitions is partially removed with the consequence that the probabilities of singlet-triplet absorptive transitions, singlet-triplet intersystem crossing from the lowest excited singlet state, phosphorescence and triplet-singlet intersystem crossing from the lowest triplet to the ground state are substantially increased. However, the external heavy atom effect also increases the rate of radiationless as well as radiative deactivation of the lowest triplet state and occasionally, the radiationless process is favored over phosphorescence (2).

Room Temperature Luminescence on Solid Supports.

Solid surface luminescence analysis involves the measurement of fluorescence or phosphorescence of components adsorbed on solid materials. A variety of solid materials has been employed in analysis; these materials include silica gel, aluminum oxide, filter paper, silicone rubber, sodium acetate, potassium bromide, sucrose, and cellulose (5). However, since the early 1970s, much of the attention of solid support luminescence work has been directed towards room temperature phosphorescence (6-11).

Although phosphorescence can, in theory, be observed from gases, liquids or solids, in practice the experimental conditions required for the observation of this radiative emission make the use of gases and liquids difficult. At room temperature the long-lived triplet molecule must be rigidly held to minimize the nonradiative processes and it must also be protected from the quenching effects of oxygen. The requirements of low pressures and presence of inert gases in gaseous media make it impractical from the analytical point of view. The observation of phosphorescence in liquids is also difficult, since intermolecular collisional quenching contributes to weak or nondetectable phosphorescence and solutions must be thoroughly deoxygenated (1).

Before the development of solid-substrate RTP, in order to observe phosphorescence, liquid coolants, such as liquid N_2 , were necessary; the phosphor was immobilized in a frozen matrix. However, there are many problems associated with observation of phosphorescence at low temperature. Some of these problems include the need for cryogenic equipment, the problems related to the introduction of the sample and matrix effects (cracking, snowing, nonuniformity in the microenvironment).

It has been found that only certain substrates are useful for inducing RTP from adsorbed organic compounds. Filter paper is the most widely used solid substrate to date for inducing RTP. Because several aspects of solid surface RTP are not yet understood, solid substrates for inducing RTP are still chosen somewhat empirically (12). Some of the solid substrates which have been found to induce RTP have included chalk, sodium acetate, polyacrylic acid treated filter paper, polyacrylic acid-sodium halide mixtures and, boric acid. There have been two major reviews on the area of RTP. Vo-Dinh (1) and Hurtubise (5) have both given

extensive details of the experimental needs for solid-surface RTP. Solvents, drying time, moisture, background luminescence, and variation of the properties of similar solid substrates are some of the more important experimental aspects. It has been found that solvents can have an important effect on whether RTP is observed using solid substrates. Von Wandruszka and Hurtubise found that common alcohol solvents were useful for the observation of RTP of many compounds adsorbed on sodium acetate. They also found that when these compounds were adsorbed on sodium acetate from aprotic solvents, such as ether, acetone, dimethylformamide, and cyclohexane, no RTP was observed (13).

They postulated that the adsorption of p-aminobenzoic acid (PABA) on sodium acetate was preceded by partial neutralization with dissolved sodium acetate in alcoholic solutions. The PABA anion formed had a strong tendency to adsorb on the surface, forming the sodium salt. This was supported by the strong RTP of the sodium salt of PABA when it was adsorbed on sodium acetate suspended in an ethanolic solution of the sodium salt of PABA. No RTP signals were observed for PABA and the sodium salt of PABA dissolved in acetone or dimethylformamide. They suggested that ion pairing and the formation of conjugated species in these solvents probably were responsible for the lack of RTP (13).

Background luminescence from solid surfaces can cause problems in both solid-surface fluorescence and phosphorescence. There has been much effort to reduce the luminescent background which is due to impurities present in the hemicellulose and/or lignin residues present in the cellulose fiber (14). Some of the approaches have included soaking in ethanol. However, this approach is not always satisfactory. Lue-Yen Bower et al. reported several pretreatment methods for substrates. These

included baking for various periods at various oven temperatures, eluting with polar and nonpolar solvents, and irradiating with several light sources (15). They found no combination of substrates and pretreatments that would enhance the signal-to-noise ratio. Bateh and Winefordner (14) conducted further studies on the treatment of cellulose materials as substrates in RTP. The treatments involved soaking techniques using dioxane-water, diethylenetriamine-pentacetic acid, water, ether, boiling water, sodium hydroxide, and periodic acid. Bateh and Winefordner concluded that pretreatment would improve the adsorption characteristics of filter paper but that the phosphorescence background probably would not be reduced substantially. Later, McAleese and Dunlap (16) showed that the phosphorescence background of filter paper (Whatman 3MM) could be reduced considerably by exposing the filter paper to 285-nm radiation or white light from a xenon lamp for 57 hours. The authors claimed that the calculated detection limit of PABA adsorbed on the illuminated filter paper had been reduced to approximately 0.5 pg which was 200 to 200,000 lower than previously had been reported on solid supports (16).

Water also has been found to have a deleterious effect on the RTP of many compounds. Schulman and Parker showed with filter paper that the presence of moisture favored increased quenching of RTP by aiding the transport of O_2 into the sample matrix (17). Thus it is important to dry the sample and substrate prior to making measurements (12).

Hydrogen bonding has been proposed as one of the mechanisms for inducing RTP from solid supports (which include sodium acetate, filter paper and silica gel). For instance, silica gel has been shown to be useful for inducing RTP from organic compounds under certain conditions (5,18-23). Ford and Hurtubise excited terephthalic acid (TPA) adsorbed

on dried silica gel with shortwave ultraviolet radiation after the silica gel chromatoplate was submerged in n-hexane. They assumed n-hexane would not disrupt hydrogen bonding between the adsorbate and adsorbent. Their assumption was valid because RTP from the submerged chromatoplate was observed with little or no loss of intensity. However, when the same dried chromatoplate was placed in distilled water, the RTP of TPA was immediately quenched, presumably because of the disruption of hydrogen bonds between TPA and silica gel surface. The RTP reappeared after the chromatoplate was removed from the water and dried (5,18).

The RTP of pyridine type nitrogen heterocycles adsorbed on silica gel was also investigated by Ford and Hurtubise (5,19). Benzo[f]quinoline was used as a model compound on several silica gel samples. They used luminescence, reflectance, and infrared spectroscopy to obtain a better understanding of the analytical conditions needed for the observation of strong RTP. The results showed that silica gel chromatoplates, containing a polymeric binder with carboxyl groups, were the best samples for inducing strong RTP from benzo[f]quinoline. It was shown that the polymer itself was essential for inducing strong RTP. Their results clearly indicated that enhanced RTP signals were obtained only when the binder was in its acidic form. Thus, acidic solutions of the nitrogen heterocycles had to be spotted on the chromatoplates for strong RTP signals. Ford and Hurtubise proposed hydrogen bonding between the pi-electron systems of the protonated nitrogen heterocycles and the carboxyl groups of the polymeric binder. Since the acidic polymer is a stronger acid than the silanol groups from silica gel, the carboxyl groups would be expected to form stronger hydrogen bonds with the pi-electron system of nitrogen heterocycles and therefore hold the compounds more rigidly to the surface.

In addition, the protonated nitrogens of the nitrogen heterocycles could form hydrogen bonds with the carbonyl oxygens of the carboxyl groups of the binder.

Ford and Hurtubise also showed that polyacrylic acid-NaCl mixtures induced strong RTP from nitrogen heterocycles indicating that silica gel was not necessary for inducing RTP. As additional evidence for the hydrogen bonding mechanism, the hydrocarbon analogue of benzo[f]quinoline, phenanthrene, was adsorbed on a 5% polyacrylic acid-NaCl mixture; the phenanthrene also exhibited a moderate RTP signal when spotted from an acidic ethanol solution onto silica gel chromatoplates which contained the salt of an acidic binder. Their results suggested the excess acid converted carboxylate groups to carboxyl groups which then interacted strongly with phenanthrene. Because phenanthrene has no heteroatom, they concluded the main interaction responsible for the enhanced RTP observed from this compound was hydrogen bonding between the carboxyl groups of the binder and the pi-electron system of phenanthrene (5).

Within the last few years, various types of organized media have been used with solid-surface luminescence techniques. Alak et al. reported enhancements in the fluorescence of twelve dansyl amino acids and four polycyclic aromatic hydrocarbons (PAHs) spotted on five common TLC stationary phases when various surfactant and cyclodextrin spray reagents caused luminescence enhancements on some stationary phases but not others. The reagents did not affect all compounds to the same degree indicating that qualitative information could be obtained in some cases. It was found that in many cases, the detection limits of dansyl glycine were lowered and the linear dynamic range increased when various types of organized media were used. The largest luminescence increase for a

compound spotted on silica gel was for pyrene where a 47-fold increase in signal was obtained when it was sprayed with sodium cholate (24).

Cyclodextrin induced solid-surface room temperature luminescence of several compounds was obtained when α -cyclodextrin was mixed with NaCl (25). Bello and Hurtubise found that the room temperature luminescence (RTL) of several compounds had relatively wide linear dynamic ranges extending over several hundred nanograms. The limits of detection (LODs) were found to be in the nanogram and subnanogram region (25,26). Many of the enhancements observed using the α -cyclodextrin-NaCl mixture can be attributed to the interactions of α -cyclodextrin-analyte interactions. In many cases, the analyte was either completely or partially included in the cavity of the cyclodextrin (27).

Vo-Dinh and Alak (28) investigated the enhancement of RTP of anthracene when the compound is adsorbed on cyclodextrin-treated filter paper. The results showed that β -CD treatment induced a significant increase in anthracene RTP emission, which is normally extremely weak. It was also found that α -CD did not produce any strong RTP enhancement, while, γ -CD produced relatively lower enhancement than β -CD did.

Luminescence in Organized Media

Both fluorescence and phosphorescence of many aromatic molecules have been observed in various types of organized media. Organized media have the ability to solubilize, compartmentalize, and concentrate (or separate) reactants/analytes. They can also alter the effective microenvironment (acidity, polarity, fluidity) about associated solutes, alter quantum efficiencies, alter chemical and photophysical pathways and rates, and alter the position of chemical equilibria (29). The fluorescence of many molecules in organized media is found to be greatly

enhanced when compared to that observed in homogeneous solution. The factors responsible for this increased fluorescence have been ascribed to the shielding of the molecule from vibrational quenching by the hydrogen-bond structure of water, the increased local viscosity about the analyte binding sites with a concomitant reduction of oxygen quenching, the reduction in the accessibility of solvent-borne quenchers to actual contact with the analyte, the reduction of other quenching/deactivation modes due to a more ordered local environment, and/or the alteration of photophysical rates, among others. The net effect is that the organized media afford protection to the excited singlet state so that the radiative process can favorably compete with the quenching deactivation processes (29-31).

Similarly, organized media also have provided the opportunity to observe phosphorescence at room temperature in fluid solution. Normally, phosphorescence of organic compounds has been observed only in the gas phase, in rigid media, at cryogenic temperatures (32), or more recently, on solid substrates (1,5). The observation of phosphorescence in organized media is due to an effective environment favorable to the existence of excited triplet states so that the radiative phosphorescence process can successfully compete with the nonradiative and quenching processes. The triplet state is in some fashion protected from oxygen quenching in organized media (32,33).

Amphiphilic molecules (also known as surfactants or detergents) contain distinct hydrophobic and hydrophilic regions. The combination of pronounced hydrophobic acid and hydrophilic properties within one molecule gives these molecules unique properties on dissolution in water (33). A simple typical surfactant has the structure, R-X, where R is a long-chain

hydrocarbon of 8-18 atoms, and X is the polar (or ionic) head group. Depending on X, surfactants can be classified as nonionic, cationic, anionic or zwitterionic. Micelles are formed when the amphiphilic molecules reach an appropriate concentration (critical micelle concentration, c.m.c.) and aggregate spontaneously. The c.m.c. depends on the surfactant structure and on experimental conditions. The surfactant monomers are in dynamic equilibrium between the micelles and the bulk. Micelles are thermodynamically stable and easily reproducible (33).

Micellar-enhanced fluorescence is another important technique, particularly because the emission intensity of analytes is usually many times greater than in the corresponding homogeneous media. Plots of relative fluorescence intensity vs. analyte concentration usually show a longer range of linearity in micelles compared to that observed in homogeneous (e.g. ethanol) media (31).

Although fluorescence is greatly enhanced in micelles, few analytical applications to date have been performed. Micellar-sensitized determinations of amino acids after derivatization with dansyl chloride and Roth's reagents, o-phthaldehyde-2-mercaptoethanol (OPT) have been reported. The measured detection limits of dansyl-glycine in micellar media are 12-20 times less than those obtained in water, whereas the increase in sensitivity is about 10-fold for the (OPT) derivative of lysine (34). De Moreno and Smith reported fluorescence enhancement factors of 1.76 to 8.9 for the drugs apomorphine (APO), and N-n-propyl-nor-apomorphine (NPA), and a series of model hydroxybiphenyls (35). Enhancement factors from 2 to 150 have been observed for aromatic hydrocarbons in solutions containing micelles (36-38). Although micellar

spectrofluorimetry seems to be particularly suitable for the determination of organic compounds, its use in inorganic analysis has also been reported. The use of micelles is particularly effective when the inorganic species can form fluorescent compounds (32). Some of the recently published work concerns, for example, the determination of rare earth metal ions as β -diketone/tri-n-octylphosphine oxide (TOPO) complexes in the presence of nonionic surfactants (39,40), and the improved determination of aluminum as a complex with morin using ethylene oxide/propylene oxide nonionic condensates (41).

With the exception of a few isolated reports (42-44), the observation of phosphorescence in a fluid solution at room temperature was generally not observed until the early 1970s. Vander Donckt, Matagne and Sapir reported the observation of RTP of aromatics in fluid dimethylmercury (45). They attributed the observation of RTP of these compounds to the use of a spin-forbidden transition enhancer, dimethylmercury. Later, Turro et al. (46) reported the observation of RTP of bromonaphthalenes in nitrogen purged solutions of acetonitrile and 1,2-dibromoethane. The room temperature phosphorescence of 4H-pyran-4-thione (PT) in inert perfluoroalkane solvents has been reported (47). A phosphorescence quantum yield of PT was reported to be 0.33 to 0.47 depending on the mode of excitation.

Room temperature phosphorescence also has been observed from colloidal or microcrystalline suspensions of aromatic molecules (48,49). It was found that this method represented the simplest means of observing RTP since sample preparation was minimal, deaeration was unnecessary, and temporal discrimination was not required. It has been suggested that the microcrystals act in a manner analogous to solid-substrate RTP in that the

quenching pathways that normally operate at room temperature are minimized. The microcrystals serve as their own substrate and provide stability to the triplet state. Since the solute is present as a solid in suspension, diffusion of molecular oxygen through the solute is not possible, thus preventing oxygen quenching (49).

Triplet state energy transfer in fluid solution is a well documented and a relatively common phenomenon (50-52). The process involves energy transfer from a donor molecule to the triplet state of an acceptor. If the acceptor molecule then emits phosphorescence radiation, the donor is said to sensitize the emission of the acceptor (53). The two most commonly used acceptors are 1,4-dibromonaphthalene and biacetyl. Sensitized RTP in fluid solutions has been found to be quite sensitive. For example, the limit of detection of 1,4-dibromonaphthalene in n-hexane at room temperature was found to be 5.4×10^{-9} M (54), and the limits of detection of a series of polychloronaphthalenes (PCNs), via sensitized room temperature phosphorescence of biacetyl in an azeotropic acetonitrile/water were on the order of 10^{-8} to 10^{-9} M (55). DeLuccia and Cline Love investigated the effect of cyclodextrin cavity size on the sensitization of the RTP of biacetyl. They found that the phosphorescence intensity of biacetyl increased as the size of the cyclodextrin cavity increased. This increase in biacetyl intensity is because the reactants were better included in the larger cavity and better positioned to increase the rate of triplet-triplet energy transfer (56).

Micelles also have provided the opportunity to observe phosphorescence in fluid solution. In mixed micellar solutions of Ag/Na and Tl/Na dodecyl sulfate (Tl/Na DS), strong phosphorescence of many aromatic molecules was obtained. In the absence of heavy atoms, no MS-

RTP is observed. These hydrophobic molecules are attracted to the oil-like environment of the micelle and, in so doing, are placed in proximity of the heavy atom counterions whose local concentration is estimated to be 3 to 5 M (53, 57-59). The limits of detection obtained were comparable to that obtained at 77 K.

One of the problems associated with the observation of phosphorescence in fluid solutions is the presence of oxygen which quenches phosphorescence. Many methods have been used for deoxygenating solutions. Some of these include bubbling the solutions with nitrogen or other inert gas, repeated freeze-thaw cycles, or preparation of samples in a vacuum. Quite recently, a novel method of sample deoxygenation, based on the principle of oxygen diffusion through a permeable membrane in conjunction with chemical scavenging of oxygen, has been described (60). However, this method has been found to be time consuming. Diaz Garcia and Sanz-Medel have described a technique that utilizes sodium sulfite as an oxygen scavenger in micellar media, which has proved to be very convenient (61).

Luminescence also has been observed in cyclodextrins. These molecules are macrocyclic carbohydrates that form a doughnut-like structure, the size of the doughnut "hole" being determined by the number of units in the ring (62). Cyclodextrins form inclusion complexes with many molecules in fluid solution if the steric properties of the molecule permit it to at least partially enter the cyclodextrin cavity (53). If a heavy atom reagent, such as dibromoethane is also present in solution, intense RTP signals can be induced (63-65). Cyclodextrins have also been used in the fluorimetric determination of metal-chelates. The use of the inclusion complex of 1-amino-4-hydroxy-anthraquinone (AHA) in the internal cavity of β -cyclodextrin is compared with AHA alone as a ligand for the

spectrofluorometric determination of beryllium. The organizing ability of the cyclodextrin medium and the protection of the ligand from the microenvironment confer increased sensitivity, selectivity, and detection limits, and allow the determination of 10-70 ng/ml beryllium, compared to 60-500 ng/ml obtained in the absence of the cyclodextrin (66).

Goals of Dissertation

It is the goal of this dissertation to use various types of organized media to enhance and to alter the luminescence properties or excitation-deexcitation pathways of different types of molecules both on solid-substrate and in solution.

First, the effects of surfactants of different ionic nature on the room temperature phosphorescence properties of two different classes of compounds, nonpolar and polar, are explored in order to enhance the intensities and, hence, obtain lower limits of detection.

Secondly, surfactant vesicles with magnetite particles encapsulated in them (providing an internal magnetic field) are used to try to alter and enhance various types of luminescence properties of several molecules.

Thirdly, cyclodextrins of various cavity sizes are used with alcohols (brominated and nonbrominated) as a third component to enhance various luminescence properties of different molecules. Cyclodextrins are probably the most specific form of organized media based on their ability to effectively discriminate between molecules based on size and shape. This type of organized media potentially can be used to discriminate between different types of molecules, or even positional isomers without prior separation.

CHAPTER 2

PAPER SUBSTRATE ROOM TEMPERATURE PHOSPHORIMETRY ENHANCED BY SURFACE-ACTIVE AGENTS

The observation of phosphorescence at room temperature was first reported by Roth (67) on cellulose filter paper. Shortly after, Schulman and Walling (6,7) reported room temperature phosphorescence from a variety of ionic aromatic compounds adsorbed on paper, silica, alumina, and other supports.

Room temperature phosphorimetry on solid substrate, particularly on cellulose filter paper has developed into a sensitive, selective, and simple method for the analysis of small amounts of many organic compounds. Organic compounds analyzed by room temperature phosphorimetry include drugs (68-70), pesticides (71-73) and other compounds of environmental and biomedical interest (74). Two major reviews of the field have been made by Hurtubise (5) and Vo-Dinh (1).

Over the years, there have been many studies to explain the nature of the interactions between the phosphor and support in an attempt to come up with the "ideal" substrate. From the studies of Roth (67) and Schulman and Walling (6,7), initially it was thought that only polar or ionic organic compounds exhibited room temperature phosphorescence when adsorbed on a suitable material such as silica, alumina or cellulose and dried; under these experimental conditions, nonionic forms of the compounds showed little or no phosphorescence (7). Schulman and Walling suggested that surface adsorption of the phosphor molecules held them rigidly enough

to restrict collisional deactivation of the triplet state. Subsequent studies have shown that molecules capable of strong adsorption interactions with the support material generally prove to be the most intense phosphors at room temperature (9,10,13,17,75).

In a study by Wellons et al. (9), a trend was noted between compounds with the most ionic sites and the efficiency of room temperature phosphorescence from the compounds adsorbed on paper. Schulman and Parker have shown that reducing the polarity of the adsorbent can drastically decrease the intensity of room temperature phosphorescence from ionic compounds. This was demonstrated by comparing the room temperature phosphorescence of compounds adsorbed on filter paper with those of the same compound adsorbed on similar filter paper which had been silanized to reduce polarity. A 90% reduction in phosphorescence intensity was reported for sodium 1-naphthoate upon switching from a regular paper support to a silanized paper support (17). From these experiments, Schulman and Parker suggested that hydrogen bonding of the phosphor to the support material might serve as an important means of restricting collisional deactivation of the phosphor in some cases (17).

These same studies by Schulman and Parker showed a progressive decrease in the level of room temperature phosphorescence for samples exposed to atmospheres of increasing humidity. These results indicated that moisture might be competing with the phosphor molecule for hydrogen bonding sites on the adsorbent, thus increasing the mobility of the phosphor and the chances of collisional deactivation (17).

Room temperature phosphorescence also has been observed from several nonpolar polynuclear aromatic hydrocarbons (PNA) adsorbed on paper (76-80) or on sodium acetate (78). However, in order to observe room

temperature phosphorescence from the PNA, it was necessary to add a heavy atom perturber such as silver or thallium nitrate to the sample. Enhancement of the phosphorescence quantum efficiency by the heavy-atom effect might be one reason for the observation of room temperature phosphorescence from these compounds. It has been suggested that the observation of room temperature phosphorescence from these PNA compounds is due to the formation of polar π -complexes between the aromatic compound and the heavy atom perturber. In this case, it is suggested that adsorption of the polar π -complex could provide protection against collisional deactivation (78).

Later on, McAleese and Dunlap (81) provided evidence that observation of room temperature phosphorescence on solid substrate could not be due to immobilization of the phosphor due to a simple hydrogen bonding mechanism. They suggested that a matrix isolation mechanism effect could be responsible for the observation of room temperature phosphorescence on cellulose filter paper. They suggested that the phosphor molecule penetrated the fibers of the paper and was subsequently entrapped between cellulose chains. Cellulose undergoes considerable swelling in the presence of strongly polar agents such as water and aqueous solutions (82). Swelling results from the formation of hydrogen bonds between the water molecules and the hydroxyl groups of cellulose which opens up new areas in the fibers into which more water can enter (81,82). Thus, the swelling would enable the penetration of phosphors into submicroscopic pores in the fibers. When cellulose is dried, the fibers collapse and nearly all of the water-to-cellulose hydrogen bonds are replaced with cellulose-to-cellulose bonds (82).

Later on, in an effort to further understand the physical and

chemical interactions of the substrate with the analyte and/or the heavy atom, Andino et al. analyzed the surface of Whatman No. 1 filter paper by X-ray photoelectron spectroscopy (83). Andino et al. found no evidence of a strong chemical interaction of iodide ions with the cellulose fiber; in addition, no evidence from the XPS studies of a strong chemical interaction between the heavy atom and phosphor spotted on the surface of the papers was found. They found that both the heavy atom and luminescent molecules did indeed penetrate the bulk of the paper, but the heavy atoms or ions seemed to exceed that of the organic compound (83).

De Lima et al. modified the surface of Whatman No. 1 filter paper by the use of surface-active agents, and studied the extent of penetration of the compounds into the bulk of the filter paper. The results obtained from the surface analysis were correlated with the observed effects of the surfactants on the phosphorescence of carbaryl (84). It was found by XPS studies that both TlDS and AgDS are better retained on the surface of the paper than their corresponding nitrate salts. The ratios of silver and thallium increased by factors of 7.6 and 69, respectively, when the surfactant salts of the ions are used in place of the nitrate salts. The observed increases in the ratios of silver and thallium correlate well with the 7-fold and with an order of magnitude of the 9.5-fold increase in the room temperature phosphorescence intensities of carbaryl in the presence of AgDS and TlDS, respectively compared to the intensities obtained when AgNO_3 and TlNO_3 are used as enhancers (84). It was suggested that the increase in phosphorescence intensities of carbaryl was due to the increased availability of the heavy atom enhancer at the surface.

Paper Substrate Room Temperature Phosphorimetry of Polyaromatic Hydrocarbons Enhanced by Surface-Active Agents

Surface-active agents (surfactants, detergents) are amphiphilic molecules which contain distinct hydrophobic and hydrophilic regions. The combination of pronounced hydrophobic and hydrophilic properties within one molecule gives these molecules unique properties on dissolution in water. A simple typical surfactant has the structure R-X, where R is a long-chain hydrocarbon of 8-18 atoms, and X is the polar (or ionic) head group. Depending on the nature of X, surfactants can be classified as nonionic, cationic, anionic or zwitterionic (31,85). In this study, the effect of the presence of anionic, nonionic and cationic surfactants on the paper substrate room temperature phosphorimetry (PS-RTP) of several PAHs and carbazole is evaluated. The enhancement and depressing effects, spectral shifts, changes in phosphor lifetimes, and photostability of both the analytes and phosphor impurities of the paper are discussed. Analytical figures of merit are also given.

Experimental

Apparatus. Phosphorescence spectra were obtained with a Perkin-Elmer LS-5 luminescence spectrometer interfaced to a Model 3600 data station. Unless otherwise stated, excitation and emission bandpasses were set at 10 and 5 nm. Delay and gate times were set at 0.03 and 9 ms, respectively. A previously described laboratory-built sample compartment was used (86).

Chemicals. Hexane, heptane, methanol, 1-pentanol, Brij 35, dodecyltrimethylammonium chloride (DTAC), sodium decylsulfate (SdS), sodium dodecylsulfate (SDS), and reagent grade thallium (I) nitrate were used as received. Aqueous solutions were made with Nanopure deionized

Table 2-1. List of Reagents and Materials.

Reagents/Materials	Manufacturers/Suppliers
acenaphthene	Eastman Kodak Co.; (Rochester, NY)
acetone (Spectrograde)	" " " "
acetophenazine maleate	Schering Co.; (Kenilworth, NJ)
p-aminobenzoic acid	Eastman Kodak Co.; (Rochester, NY)
2-aminonaphthalene	Aldrich Chemical Co.; (Milwaukee, WI)
7,8-benzoflavone	Eastman Kodak Co.; (Rochester, NY)
biphenyl	" " " "
m,m'-bitoluene	Pfaltz & Bauer, Inc. (Stamford, CT)
Brij 35	Aldrich Chemical Co.; (Milwaukee, WI)
1-bromonaphthalene	" " X " " "
2-bromonaphthalene	" " " " "
2-bromoethanol	" " " " "
carbazole	Matheson, Coleman & Bell (Norwood, OH)
p-chlorobiphenyl	Alfa, Morton Thiokol, Inc. (Danvers, MA)
chloroform (spectrograde)	Eastman Kodak (Rochester, NY)
chlorpromazine hydrochloride	Smith-Kline & French Labs (Philadelphia, PA)
α -cyclodextrin	Aldrich Chemical Co., (Milwaukee, WI)
β -cyclodextrin	" " " " "
γ -cyclodextrin	" " " " "
1,2-dibromoethane	" " " " "
2,3-dibromopropanol	" " " " "
dihexadecyl phosphate	Sigma, (St. Louis, MO)
2,3-dimethylnaphthalene	Aldrich Chemical Co., (Milwaukee, WI)
dodecyl trimethylammonium chloride	Eastman Kodak Co., (Rochester, NY)
96% ethanol	Florida Distillers Co., (Lake Alfred, FL)
fluoranthene	Chem. Service (Media, PA)
fluorene	Aldrich Chemical Co., (Milwaukee, WI)
fluphenazine hydrochloride	Schering Co., (Kenilworth, NJ)
heptane	American Burdick and Jackson, (Muskegon, MI)
hexane	" " " "
hydrochloric acid	Fisher Scientific (Fairlawn, NJ)
isoquinoline	Chem. Service (West Chester, PA)
lepidine	Aldrich Chemical Co., (Milwaukee, WI)
methanol	American Burdick and Jackson (Muskegon, WI)
magnetite	Matheson, Coleman & Bell (Norwood, OH)
naphthalene	Aldrich Chemical Co., (Milwaukee, WI)
1-naphthol	Fisher Scientific Co., (Fairlawn, NJ)
9-nitroanthracene	Aldrich Chemical Co., (Milwaukee, WI)
2-nitrofluorene	" " " " "
1-nitropyrene	" " " " "
perphenazine	Schering Co., (Kenilworth, NJ)
phenanthridine	Aldrich Chemical Co., (Milwaukee, WI)
phenanthrene	" " " " "
1-pentanol	" " " " "
phenazine	" " " " "

Table 2-1. continued.

Reagents/Materials	Manufacturers/Suppliers				
prochlorperazine edisylate	Smith-Kline & French Labs, (Philadelphia, PA)				
promazine hydrochloride	Wyeth Labs, (Philadelphia, PA)				
1-propanol	Fisher Scientific Co., (Fairlawn, NJ)				
2-propanol	"	"	"	"	"
pyrene	Aldrich Chemical Co., (Milwaukee, WI)				
1-pyrenebutyric acid	"	"	"	"	"
1-pyrenecarboxaldehyde	"	"	"	"	"
quinoline	Mallinckrodt				
retene	Eastman Kodak Co., (Rochester, NY)				
serentil	Boehringer, Ingelheim				
sodium decyl sulfate	Eastman Kodak Co., (Rochester, NY)				
sodium dodecyl sulfate	"	"	"	"	"
sodium sulfite (anhydrous)	Fisher Scientific Co., (Fairlawn, NJ)				
sodium tetradecyl sulfate	Eastman Kodak Co., (Rochester, NY)				
sodium heptane sulfonate	"	"	"	"	"
o-terphenyl	City Chemical Corp., (NY)				
thallium(I)nitrate	Fisher Scientific Co., (Fairlawn, NJ)				
thiopropazate hydrochloride	Searle & Co.,				
thioridazine hydrochloride	Sandoz Pharmaceuticals,				
triflupromazine hydrochloride	Squibb Institute for Medical Research				
trimeprazine tartrate	Smith-Kline & French Labs, (Philadelphia, PA)				
water (nanopure)	Barnstead System of Sybron (Boston, MA)				
Whatman DE-81	Whatman Laboratory Products, Inc. (Clifton, NJ)				
Whatman No. 1	"	"	"	"	"
Whatman No. 4	"	"	"	"	"
Whatman No. 50	"	"	"	"	"
Whatman P-81	"	"	"	"	"

water (Barnstead Sybron Corp., Boston, MA). Fluorene, 2,3-dimethylnaphthalene were recrystallized three times from ethanol, and o-terphenyl was recrystallized three times from methanol. The other PAHs and carbazole used in this study were used as received. Whatman No. 1, Whatman DE-81 (anion-exchange), and Whatman P-81 (cationic exchange) filter paper were used as solid substrates. Table 2-1 indicates the sources of these chemicals and materials.

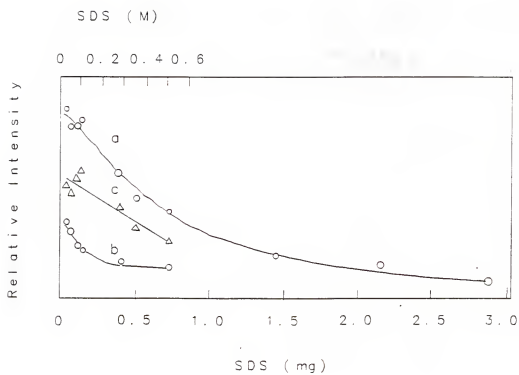
Procedure. Stock solutions of the PAH's were prepared by dissolving 1-5 mg in 0.5 ml of heptane and 0.5 ml of 1-pentanol and diluting to 25 ml with aqueous 0.5 M SDS. Similar amounts of the PAHs were dissolved in hexane and in aqueous solutions of the other surfactants, using an ultrasonic bath (Cole Palmer, Chicago, IL). Carbazole was dissolved in pure methanol.

Strips of Whatman No. 1 filter paper were held on the sample holder by means of a blackened metallic frame and two screws. A 6 x 6 mm² square window limited the exposed surface. The reagents and samples were spotted on the paper in the center of the window using 5- μ l Hamilton syringes. The paper was then dried for 15 min in an oven at 110°C and allowed to cool in the sample compartment for 10 min under a stream of nitrogen which was previously dried over CaSO₄. The sample holder position was optimized for each sample (86). o-Terphenyl was chosen as the model compound for most of these studies. In these experiments, the excitation and emission wavelengths used were 265 and 474 nm, respectively.

Results and Discussion

Order of Addition of the Reagents. When the paper substrate was spotted with 2 μ l of 0.25 M TiNO₃ and 5 μ l of 16 μ g/ml of o-terphenyl solution in hexane, the average RTP intensities were 170 ± 10 and $35 \pm$

Fig. 2-1. Influence of SDS concentration: (a) paper spotted with 2 μ l of 0.25 M TiNO_3 and 5 μ l of 16 $\mu\text{g/ml}$ o-terphenyl in SDS at various concentrations; (b) blanks; (c) net signals.

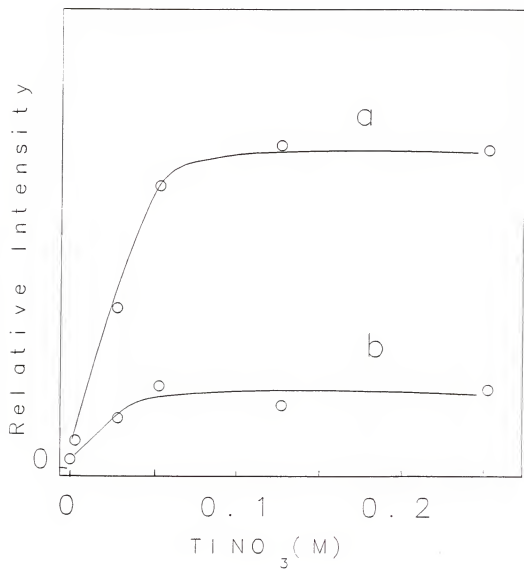


5 for the analyte and paper background, respectively (four trials). No significant differences were observed when the reverse order of reagent addition was used. When using a 16 $\mu\text{g}/\text{ml}$ o-terphenyl solution in 0.5 M SDS instead of a hexane solution, an enhancement of the net signal of about 100% was observed, with the analyte and blank intensities being 375 ± 15 and 90 ± 10 , respectively. Values of about 20% lower were obtained when the micellar solution was spotted before TlNO_3 .

Surfactant and Thallium Concentrations. In order to study the influence of the SDS concentration, a solution of 160 $\mu\text{g}/\text{ml}$ o-terphenyl in 0.5 M SDS was prepared. Dilutions containing 16 $\mu\text{g}/\text{ml}$ o-terphenyl and different concentrations of SDS were made. Two microliters of 0.25 M TlNO_3 and 5 μl of the micellar solutions were spotted on the paper. The results are shown in Figure 2-1. The three points with the highest amounts (equivalent to molar concentrations above 0.6 M) of SDS were obtained by spotting the analyte solution several times and drying the paper between applications. In this case, a 16 $\mu\text{g}/\text{ml}$ solution of o-terphenyl in 0.5 M SDS was diluted 2, 3, and 4 times with 0.5 M SDS and spotted the same number of times. The emission intensity decreased as the surfactant concentration was increased. An five times enhancement factor of the net signal was obtained with 0.05 M SDS. Smaller concentrations of the surfactants were not tried due to the limited solubility of o-terphenyl in aqueous micellar solutions containing a low concentration of SDS. Surfactant concentrations of 2% (corresponding to 0.07 M SDS) were used in all the following experiments.

As shown in Figure 2-2, the phosphorescence intensity increased rapidly with thallium concentration, but no further increase was achieved beyond 0.1 M, where saturation of the substrate seemed to have been

Fig. 2-2. Influence of TlNO_3 concentration: (a) paper spotted with $2\ \mu\text{l}$ of TlNO_3 of various concentrations and $5\ \mu\text{l}$ of $16\ \mu\text{g/ml}$ o-terphenyl on $0.5\ \text{M}$ SDS; (b) blanks.



reached. A thallium concentration of 0.25 M was used for the remainder of the experiments.

Influence of the Surfactant Character. Triplicate experiments were performed in the presence of TlNO_3 using surfactants with 12-carbon atom linear aliphatic tails but with various polar head characters. The polar head groups were sulfate and trimethylammonium in the anionic SDS and cationic DTAC, respectively, and a 23-unit polyoxyethylene chain in the nonionic surfactant, Brij 35. A shorter tailed anionic surfactant, sodium decyl sulfate (SdS), was also used. As shown in Table 2-2, enhancement factors were similar for the two anionic surfactants, but drastic reductions in intensity were observed in the other two cases. In the presence of the cationic surfactant (DTAC), the spectrum of the analyte disappeared completely; the phosphorescence intensities of the blanks were similarly affected, increasing by a factor of 3-4 with the anionic surfactants and decreasing by a factor of 2-3 with Brij 35 and DTAC.

It is of interest to observe that the anionic surfactant enhances the phosphorescence, whereas the cationic and nonionic surfactant diminish the phosphorescence intensities of both the analytes and the blanks. A possible explanation for these effects could rely on the association of the nonpolar phosphor molecules with the nonpolar surfactant tails. The probability of finding a thallium ion increases in the proximity of a surfactant anionic head and decreases if the surfactant head is cationic. The phosphor molecules and thallium ions are probably closer to each other in the presence of an anionic surfactant and farther apart if the surfactant head is a large nonionic chain or a cationic group.

Table 2-2. Influence of the Surfactant Character

Surfactant ^a	λ_{exc} , nm	peaks found λ_{em}^b , nm	intensity ^c		enhancement factor ^e
			sample	blank	
sodium dodecyl sulfate	260	<u>450, 478</u>	472	100	4.4
sodium decyl sulfate	260	<u>450, 476</u>	560	135	5.3
Brij 35	260	<u>451, 475</u>	58	17	0.5
dodecyltrimethyl- ammonium chloride	260	<u>474^d</u>	18	14	-. ^f

^aPaper spotted with 2 μ l of 0.25 M $TiNO_3$ and 5 μ l of 8 μ g/ml of o-terphenyl in a 2% surfactant solution.

^bMost intense peak underlined.

^cIntensity at the most intense peak of the analyte.

^dNo peak observed, but this wavelength was used for the intensity measurements.

^eEnhancement factors were calculated with respect to the intensity obtained by spotting 2 μ l of 0.25 M $TiNO_3$ and 5 μ l of 8 μ g/ml o-terphenyl in hexane.

^fNo phosphorescence was observed.

Phosphorescence Spectra of Several PAHs and Carbazole. The spectral characteristics for the nine PAHs and carbazole spotted from hexane (methanol for carbazole) and aqueous 2% SDS solutions, are given in Table 2-3. When micellar solutions were used, small blue-shifts (up to 6 nm) (868 cm^{-1}) were observed in the excitation spectra of the PAHs and the bands become somewhat broader. On the other hand, spectral shifts and changes in the shape of emission spectra are negligible. These spectral blue-shifts observed for the excitation spectra could be due to a more rigid environment; this increase in rigidity could be due to better entrapment within the cellulose fibers when the analyte was spotted from the surfactant solution than when spotted from hexane. Blue-shifts have been observed in the excitation spectra when solvents of increasing polarity were used to spot samples on cellulose (81).

Influence of the various types of filter paper. The influence of the various types of surfactants on DE-81 and P-81 filter papers was examined; both these filter papers are used as ionic exchange filter papers, DE-81, an anionic filter paper, and P-81, a cationic filter paper. o-Terphenyl was used as model compound. Table 2-4 summarizes the results. As observed, there was an enhancement of ~ 2 times when SDS was used with the P-81 filter paper, and a considerable decrease in the phosphorescence of o-terphenyl was observed when DTAC was used. No enhancement in the signal of o-terphenyl was observed when the analyte was spotted from SDS and DTAC on the DE-81 filter paper; no phosphorescence was observed when DTAC was used. The structures of the filter papers used in these studies, Whatman No. 1, DE-81 and P-81 are shown in Figure 2-3. These results seem to indicate that with the exception of the case when the analyte was spotted from SDS on the P-81 filter paper, the negative or positive

Table 2-3. Paper Substrate RTP Spectral Characteristics of PAHs and Carbazole Spotted with Hexane and Micellar Solutions.

Compound	Paper spotted with hexane solution			
	λ_{exc} (nm) ^b	Half-widths (nm) ^c	λ_{em} (nm) ^b	Half-widths (nm) ^c
Biphenyl	266	29(1)	444(71), 471(100), ~495(72) _{sh}	82(3)
m,m'-Bitoluene	267	31(1)	451(73), 477(100) ~500(76) _{sh}	88(3)
Carbazole ^a	258(100)	19(2), 14(1) 291(94)	416(72), 437(100), ~462(65) _{sh}	74(3)
p-Chloro-biphenyl	268	31(1)	454(71), 481(100), ~505(76) _{sh}	91(3)
2,3-Dimethyl-naphthalene	277	43(3-4)	495(90), 515(100)	105(3)
Fluorene	271(100),	32(1) 299(48)	430(73), 459(100), ~475(78) _{sh}	80(3)
Phenanthrene	259(100),	34(2) 294(52)	472(94), 505(100), 544(55)	67(2)
Pyrene	324(76),	29(2) 337(100)	594(100), ~605(70) _{sh}	27(2)
Retene	268(100),	33(1) ~308(15) _{sh}	481(99), 514(100)	64(2)
o-Terphenyl	266	32(1)	450(78), 478(100), ~500(76) _{sh}	89(3)

Table 2-3. Continued

Compound	Paper spotted with micellar solutions ^d		
	$\lambda_{exc}(\text{nm})^b$	Half-widths (nm) ^c	Half-widths (nm) ^c
Biphenyl	261	36(1)	441(67), 472(100), -500(81) ^{sh}
m, m'-Bitoluene	262	36(1)	452(72), 478(100), -505(74) ^{sh}
Carbazole	260(100), 292(89)	20(2), 16(1)	416(74), 438(100) -462(67) ^{sh}
p-Chloro- biphenyl	264	34(1)	453(71), 482(100), -505(76) ^{sh}
2,3-Dimethyl- naphthalene	276	52(3-4)	488(88), 518(100)
Fluorene	265(100), 297(42)	32(1)	430(72), 459(100), -485(72) ^{sh}
Phenanthrene	257(100), 292(38)	35(2)	472(92), 505(100), 543(52)
Pyrene	320(78), 337(100)	31(2)	594(100) -605(71) ^{sh}
Retene	265(100), -300(26) ^h	30(1)	482(97), 514(100),
o-Terphenyl	260	36(1)	450(81), 478(100), -510(72) ^{sh}

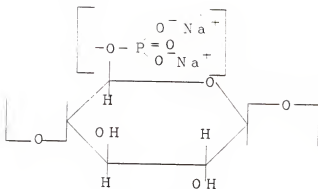
^aMethanol used instead of hexane for carbazole.^bRelative intensities in parenthesis; sh = shoulder^cIn parenthesis, number of bands involved.^d2% SDS aqueous micellar solution.

Table 2-4. Influence of Various Types of Filter Paper on the Phosphorescence of o-Terphenyl^a

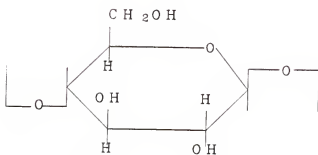
$\lambda_{\text{ex}}/\lambda_{\text{em}}$	Type of filter paper	Media	Net Relative Intensity	Enhancement factor
266/478	P-81	heptane	58 ^b	1.0
260/478	P-81	SDS	138 ^c	1.9
260/478	P-81	DTAC	10 ^d	0.2
266/478	DE-81	heptane	95 ^b	1.0
260/478	DE-81	SDS	89 ^c	0.7
260/478	DE-81	DTAC	0	-- ^e

^a2 μl of 0.1 M TiNO_3 and 5 μl of o-terphenyl in various solvents.^b4 $\mu\text{g}/\text{ml}$ of o-terphenyl in heptane.^c5.14 $\mu\text{g}/\text{ml}$ o-terphenyl in 3% SDS.^d3.6 $\mu\text{g}/\text{ml}$ o-terphenyl in 3% DTAC.^eno phosphorescence observed.

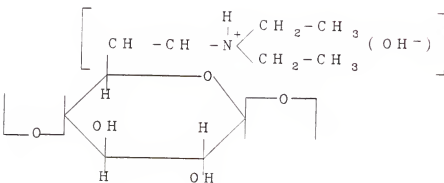
Fig. 2-3. The structures of three types of filter paper used in this study: P-81, Whatman No. 1, and DE-81.



P-81



Whatman # 1



DE-81

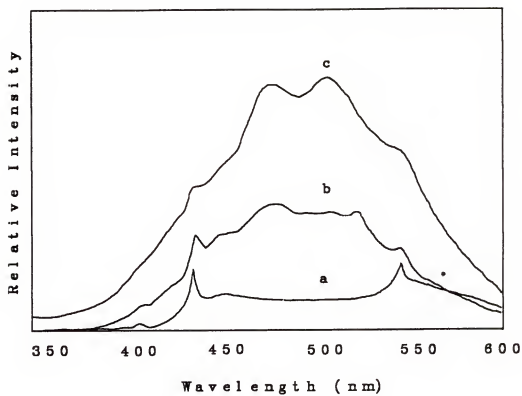
charges of the P-81 and DE-81 filter papers are interfering with the attachment of the phosphor to the filter paper.

Source of the Background. The influence of the presence of $TlNO_3$ and SDS on the emission spectra of the blanks is shown in Figure 2-4. Spectrum a, which corresponds to a paper spotted only with water, showed peaks at 404, 434 and 542 nm and a broad band with a maximum at about 450 nm. When $TlNO_3$ and SDS were successively added (spectra 2-4, b and c), the background emission increased, and new broad bands centered at about 475, 505, and 520 nm appeared. These bands are probably a result of phosphor impurities in the paper. This type of background is well documented in the literature (14,16,87).

When the background spectrum which appeared in the absence of thallium (Figure 2-4, spectrum a) was recorded by using smaller excitation and emission slits (3 nm) and shorter delay times (~ 0.01 ms), narrower peaks at the same wavelengths (404, 434, and 542 nm) along with new peaks were obtained. Because these peaks and broad band were observed when the paper was replaced with reflective surfaces such as stainless and aluminum foil, it was concluded that this background arose from the scatter of stray radiation, probably from the afterglow of the Xe flashlamp in the LS-5 fluorimeter. The wavelengths of most of the peaks agreed well with the most prominent emission lines of the Xe(II) emission spectrum (88).

Influence of Exposure to the Excitation Radiation. Both analytes and phosphor impurities of the paper can undergo photodegradation when exposed to UV radiation; a decrease in the emission intensity of all the compounds studied here was observed. Measurements of the relative decomposition rates were carried out by using the following empirical procedure.

Fig. 2-4. Influence of TlNO_3 and SDS on the background emission spectra: (a) paper spotted with 2 μl of water; (b) 2 μl of 0.25 M TlNO_3 added; (c) 5 μl of 2% SDS added to (b). The maximum of the background excitation spectra at 270 nm was used.



After the initial period of 20-30 s, which was necessary to "optimize" the sample position, the emission intensity was measured for a 1.5 min period. A plot of logarithm of the intensity versus time gave curves that were approximately linear for most of the analytes. The points were fitted to a straight line and the relative decrease in the intensity during the first minute of exposure, $-\Delta I$, was calculated. The results are given in Table 2-5. In all cases, a higher stability of the compounds spotted from micellar solutions was observed. A plot of the values of $-\Delta I$ versus the corresponding excitation wavelengths (Figure 2-5 a,b) showed an increased stability at longer wavelengths. A similar plot obtained with the blanks is given in Figure 2-5 c,d). In this case, the intensity decay was only a result of photodecomposition of the phosphor impurities. The points indicated again a better stability at longer excitation wavelengths and in the presence of SDS. The same conclusions were obtained from measurements performed with o-terphenyl (Figure 2-5 e,f).

Lifetimes. Accurate measurement of the lifetimes of the phosphor spotted on paper substrates is difficult to accomplish since the observed intensity decay can arise from several sources: phosphorescence of the analyte and the impurities, photochemical degradation of both of them, and decay of the scattered stray source radiation. However, data obtained under the same experimental conditions can be useful for comparative purposes.

Lifetimes showed a clear tendency to be shorter when SDS was present. Taking into account that in the presence of the surfactant, the photochemical degradation was slower, it is reasonable to assume that after correction for this effect, even larger differences would have been

Table 2-5. Limits of Detection, Lifetimes and Intensity Losses in the First Minute Produced by Exposure to the Excitation Radiation.

Compound	SDS not present ^a		
	LOD(ng)	Lifetime (ms)	-ΔI ^c (%)
Biphenyl	5.8	1.31	9.2
m,m'-Bitoluene	12.0	1.20	8.6
Carbazole	8.2	0.27	25.0
p-Chloro- biphenyl	4.0	2.10	3.5
2,3-Dimethyl- naphthalene	20	1.14	5.8
Fluorene	0.8	0.68	6.4
Phenanthrene	2.2	1.36	16
Pyrene	0.5	1.85	0.6
Retene	2.4	1.02	34
o-Terphenyl	1.7	1.30	8.1

Table 2-5. continued.

Compound	SDS present ^b		Enhancement factor
	LOD(ng)	Lifetime (ms)	- ΔI^c (%)
Biphenyl	3.0	1.12	7.5
m,m'-Bitoluene	3.0	1.17	4.7
Carbazole	1.0	0.28	20.0
p-Chloro-biphenyl	0.7	1.49	0.5
2,3-Dimethyl-naphthalene	2.0	1.16	3.0
Fluorene	0.3	1.01	4.2
Phenanthrene	0.9	0.88	12.0
Pyrene	0.7	1.62	-0
Retene	0.3	0.90	6.4
o-Terphenyl	0.2	0.88	2.2

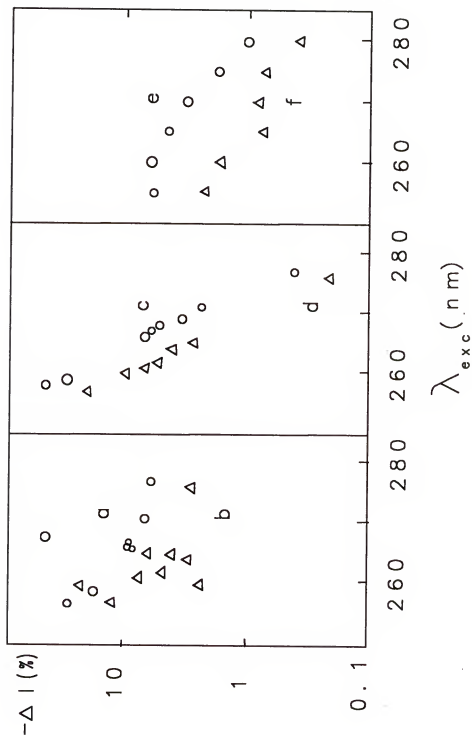
^aThe PAHs were spotted using hexane solutions. A methanol solution was used for carbazole.

^bAnalytes spotted using micellar solutions.

^cInitial intensity decrease per minute calculated from the corresponding k value.

^dCalculated from the slopes of the calibration curves.

Fig. 2-5. Influence of the excitation wavelength on the relative decrease of the RTP intensity during the first minute: circles, paper spotted with hexane solutions; triangles, paper spotted with 2% SDS solutions. For (a) and (b), values of ΔI correspond to the compounds of Table III were represented against their respective excitation maxima. For (c) and (d), blanks were excited at different wavelengths. For (e) and (f), o-terphenyl was excited at different wavelengths.



found; the lifetimes with SDS present were always shorter than in its absence.

Analytical Figures of Merit. Limits of detection (LODs) for nine PAHs and carbazole in the presence and absence of SDS are given in Table 2-5. Background noise was measured by taking 16 measurements of the blanks; the sensitivity was established from triplicate measurements at two different concentrations in the range of 5-30 $\mu\text{g/ml}$ and the LODs were calculated from these values for an $S/N = 3$.

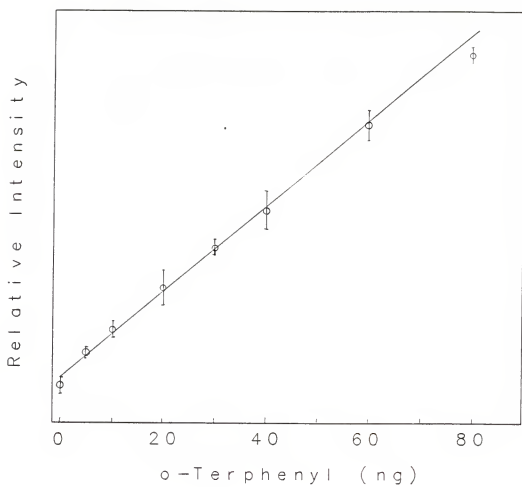
Limits of detection were about a factor of 2-10 better when 2% SDS solutions were used. The enhancement factors, calculated from the ratio of the slopes of the calibration curves in the presence and absence of the surfactant, also ranged within these values. Figure 2-6 shows the calibration curve of o-terphenyl in the presence of 2% SDS.

The relative standard deviation (RSD) was measured by using 10 independent pieces of paper spotted with TlNO_3 and 5 μl of a 8 $\mu\text{g/ml}$ solutions of o-terphenyl in 2% SDS. A value of 9% was found. Measurements performed for six independent blanks gave an RSD of 6%. All calibration curves for the polycyclic aromatic hydrocarbons had correlation coefficients for the best-fit straight line of 0.999 or better.

Paper Substrate Room Temperature Phosphorescence
of Phenothiazine Derivatives Enhanced by
Thallium (I) Nitrate and Surface-Active Agents

Paper substrate phosphorescence has the advantage of being applicable to a wide variety of molecules including both polar and nonpolar compounds. The cellulose fibers of the paper seem to offer excellent protection for the excited triplet state against nonradiative deactivation processes.

Fig. 2-6. Calibration curve for o-terphenyl in the presence of 2% SDS. Triplicate experiments were carried out.



Phenothiazine derivatives constitute an important group of pharmaceutical compounds which are mainly used as tranquilizers. The phosphorescence of phenothiazine derivatives has been studied at low temperature (89,90) but never at room temperature.

Experimental

Chemicals. Heptane, 96% ethanol, Brij 35, dodecyltrimethylammonium chloride (DTAC), sodium tetradecylsulfate (STS), sodium decylsulfate (SdS), sodium heptanesulfonate (SHS), sodium dodecylsulfate (SDS), and reagent grade thallous nitrate were used as received. Nanopure deionized water (Barnstead Sybron Corp., Boston, MA) was also used.

o-Terphenyl was recrystallized three times from methanol. p-Amino-benzoic acid, carbazole, chlorpromazine hydrochloride, prochlorperazine edisylate, trifluoperazine dihydrochloride, trimeprazine tartrate, fluphenazine hydrochloride, perphenazine, acetophenazine maleate, perphenazine, acetophenazine maleate, promazine hydrochloride, serentil, thioridazine hydrochloride, thiopropazate hydrochloride, and triflupromazine hydrochloride were used as received. The sources of these chemicals are given in Table 2-1.

An aqueous 0.25 M thallium (I) nitrate stock solution was prepared. Surfactant stock solutions (15% w/v) were prepared in a 60/40 (v/v) ethanol/water mixture; however, a 90/10 ethanol/water mixture was used for STS owing to its low solubility in water at room temperature. Stock solutions of o-terphenyl were dissolved in heptane as well as in 60/40 ethanol mixtures containing 15% SDS and in DTAC, the latter two in an ultrasonic bath. An 80/20 ethanol/water mixture was used for the stock solutions of the other compounds. Whatman no. 1, 4, 5, 50, DE-81 (anion-

exchange), and P-81 (cation-exchange) filter papers were used as solid substrates.

Apparatus. Phosphorescence measurements were made with a Perkin-Elmer LS-5 Luminescence spectrometer with a 3600 data station for obtaining the spectra. Bandpasses of 10 nm and 5 nm were used for the excitation and emission slits, respectively. Delay and gate times were 0.03 ms and 9 ms. A 305 nm cut-off filter was used in the emission side.

Procedure. The paper substrates were cut in strips and fixed to several sample holder rods, and the reagents and sample were spotted by using adjustable 10- μ l Hamilton syringes. Each paper was then dried in an oven at 110°C for 10 min and introduced into the sample compartment. The position of the analyte was adjusted until a maximum phosphorescence intensity was obtained and then dried under the stream of nitrogen for 15 min. The excitation shutter was open only during the measurements to minimize photodecomposition.

Unless otherwise noted, 2 μ l of 0.1 M thallium (I) nitrate followed by 5 μ l of the surfactant containing the analyte solution were spotted on the paper. Comparative experiments were done by spotting the corresponding solvents in the absence of thallium (I) nitrate and/or surfactant. In all cases, blanks were also run; duplicate measurements were usually made.

The phosphorescence intensities for p-aminobenzoic acid, carbazole, fluphenazine, and trifluoperazine at wavelengths of their respective main emission maxima were very noisy. The noise was probably caused by the reflection of stray radiation on the paper surface, which is particularly intense at the wavelengths of the xenon emission lines at 434 and 543 nm. For this reason, measurements of the phosphorescence intensity of these

compounds were taken 10 nm below the wavelengths of the corresponding emission maxima, except for carbazole, where the less intense maximum at 416 nm was used.

Results and Discussion

Influence of Thallium (I) Nitrate and Surfactants. In the absence of heavy atoms phenothiazine derivatives exhibited relatively intense RTP. Chloropromazine hydrochloride was chosen as a model compound to study the effect of the addition of several surfactants and thallium (I) nitrate on the RTP of phenothiazine derivatives. The results are summarized in Table 2-6.

The addition of an anionic surfactant, such as SDS, led to an enhancement of the net signal of about 3.3. When thallium (I) nitrate was added alone, the phosphorescence was almost completely quenched; surprisingly, however, when SDS and thallium (I) nitrate were both present an enhancement factor of 5.7 was obtained. Similar effects were observed when the other anionic surfactants were tested.

Enhancements were also observed when the nonionic surfactant, Brij 35 and the cationic surfactant, DTAC were added; however, in these cases, the addition of the thallium salt always quenched the phosphorescence. The different behaviors of the phosphorescence intensity upon the addition of increasing amounts of the thallium salt in the presence of SDS and DTAC are shown in Figure 2-7. When SDS was added, an initial increase followed by a flat plateau was obtained (Figure 2-7a, curve 3), but if DTAC was used instead, the intensity decreased continuously (Fig. 2-7b, curve 3). Similarly, when the SDS concentration was varied for a constant amount of the thallium salt, a broad maximum was observed (Fig. 2-8).

Fig. 2-7. Influence of TiNO_3 concentration: 2 μl of TiNO_3 and 5 μl of chloropromazine hydrochloride solution containing the surfactant on Whatman No. 1 filter paper: (a) 25 $\mu\text{g}/\text{ml}$ analyte in 3% SDS; (b) 63 $\mu\text{g}/\text{ml}$ analyte in 3% DTAC. Curves (1) blank; (2) sample; (3) net signal.

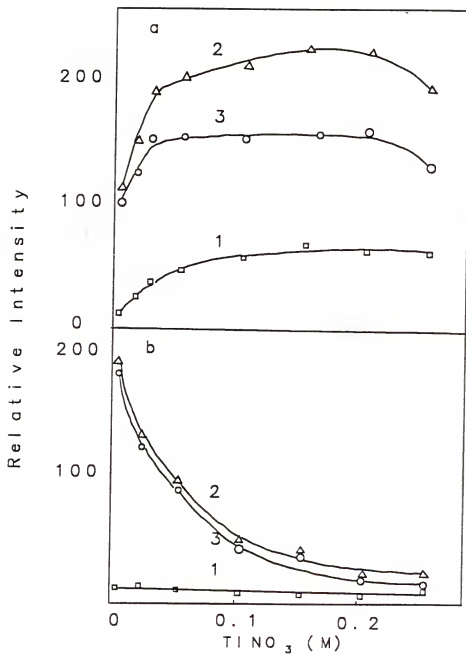


Fig. 2-8. Influence of SDS concentration: 2 μ l of 0.25 M TiNO_3 and 5 μ l of 25 $\mu\text{g/ml}$ chloropromazine solution containing different concentrations of SDS in 80/20 ethanol/water mixture were spotted on Whatman No. 1 filter paper. Curves: (1) blank; (2) sample; (3) net signal.

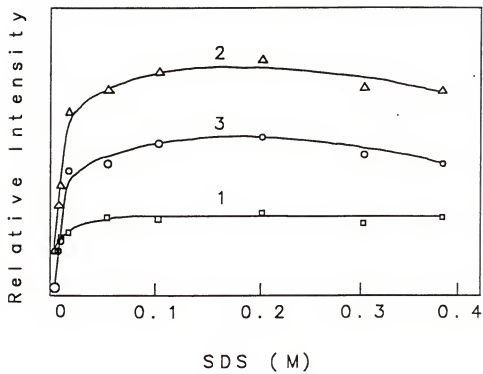


Table 2-6. Influence of Thallium(I)Nitrate and Several Surfactants on the Relative Phosphorescence Intensities of Chloropromazine Hydrochloride¹.

Type of paper	TlNO ₃ added	Surfactant added	PS-RTP relative intensities ²	Blank	Net Signal	Enhancement factor ³
Whatman no. 1	no	no	30 ⁴	7	23	1.0
	no	SDS	90 ⁴	14	76	3.3
	yes	no	32 ⁴	29	3	0.1
	yes	SDS	200 ⁴	68	132	5.7
	yes	SDS	361 ⁵	56	305	5.3
	yes	SDS	365 ⁵	60	305	5.3
	yes	SHS	305 ⁵	44	261	4.6
	yes	STS	207 ⁵	50	157	2.8
	no	Brij 35	135 ⁵	9	126	2.2
	yes	Brij 35	109 ⁵	22	87	1.5
	no	DTAC	185 ⁵	8	177	3.1
	yes	DTAC	83 ⁵	10	73	1.3
DE-81	no	no	32 ⁴	11	21	1.0
	no	SDS	56 ⁴	11	45	2.1
	yes	no	32 ⁴	25	7	0.3
	yes	SDS	75 ⁴	40	35	1.7
P-81	yes	SDS	110 ⁴	60	50	- ⁶

¹2 μ l of 0.1 TlNO₃ and 5 μ l of chlorpromazine in a 80:20% ethanol-water mixture containing the surfactant were spotted. Surfactant concentrations were 3% in all cases.

²Excitation and emission wavelengths were 260 and 522 nm, respectively. Average values from duplicated experiments are given.

³Experiments without TlNO₃ or surfactant were used as a base for calculation of the factors with each type of paper.

⁴Chlorpromazine concentration was 25 μ g/ml

⁵Chlorpromazine concentration was 63 μ g/ml as a function of time is shown in Fig. 2-9.

⁶No phosphorescence was observed.

Similar results were obtained using Whatman No. 4, 5, and 50 filter papers. However, lower intensities and no significant enhancements on addition of SDS and/or thallium (I) nitrate were found for DE-81 and P-81 filter papers. I think that the amine and phosphate groups bound to the glucopyranose units in these papers were interfering with the attachment of the phosphors or may have caused quenching of the phosphorescence from these compounds.

As shown in Table 2-7, lower enhancement factors were obtained for p-aminobenzoic acid and a somewhat different behavior was observed for the nonpolar o-terphenyl and the relatively nonpolar carbazole. An appropriate heavy atom, such as thallium, is necessary to obtain paper substrate RTP of nonpolar compounds. When the thallium salt was added, the presence of SDS produced an enhancement of the same magnitude as that observed with polar compounds, but if DTAC was used instead, the intensity dropped almost to zero. A possible explanation for this effect may be the larger distance between the phosphor and the thallium ions produced by repulsion between the latter and the positively charged heads of the surfactant. Thallium (I) salts are commonly used to observe the phosphorescence of PAHs; however, the enhancement of the phosphorescence of polar compounds by thallium ions and surfactants has not been reported previously.

Differences in the behavior of polar and nonpolar compounds were observed. The excitation spectrum of o-terphenyl exhibited a 6 nm blue shift when the surfactant was added, whereas no significant changes were observed in the shapes of the spectra and positions of the maxima for any of the polar compounds upon addition of the thallium salt and/or surfactants. Blue shifts of 2-6 nm (868 cm^{-1}) were also found for other

Table 2-7. Spectrum Maxima, Enhancement Factors and Limits of Detection for PS-RTP of PABA, Carbazole, and o-Terphenyl in the Presence of TiNO_3 , SDS and DTAC¹.

Compound, excitation and emission maxima (nm)	Reagents added	Sample	Blank	relative intensities Net Signal	Enhancement factor	LOD (ng) ²
PABA	-	141	8	133		
	TiNO_3	194	10	184	1.0	0.1
Exc: 277	SDS	245	10	235	1.4	₅
Em: 435 ³	TiNO_3 , SDS	399	20	379	1.8	0.1
	DTAC	230	9	221	2.8	0.08
	TiNO_3 , DTAC	103	8	95	1.7	₅
					0.7	₅
Carbazole	-	17	7	10	1.0	1.0
	TiNO_3	117	13	104	10.0	₅
Exc: 260	SDS	31	9	22	2.2	0.6
Em: 416(72) ⁴ , 438(100)	TiNO_3 , SDS	609	24	585	60.0	0.06
	DTAC	16	9	7	0.7	₅
	TiNO_3 , DTAC	22	17	5	0.5	₅
o-Terphenyl	-	22	13	9	1.0	0.5
Exc: 260, but 266 with SDS present	TiNO_3	142	35	107	12.0	₅
	SDS	30	16	14	1.6	0.9
Em: 450(78), 478(100)	TiNO_3 , SDS	419	64	355	40.0	0.2
	DTAC	16	13	3	0.3	₅
	TiNO_3 , DTAC	21	13	8	0.9	₅

¹Experimental conditions were the same as in Table 2-6. Whatman No. 1 filter paper was used.

²Calculated as the concentration corresponding to three times the background noise. This latter was measured by taking 16 measurements of the blanks.

³Intensity measurements were taken at 425 nm (see experimental section).

⁴This maximum was used to take the intensity measurements (see experimental section).

⁵LODs not determined for compounds under these circumstances.

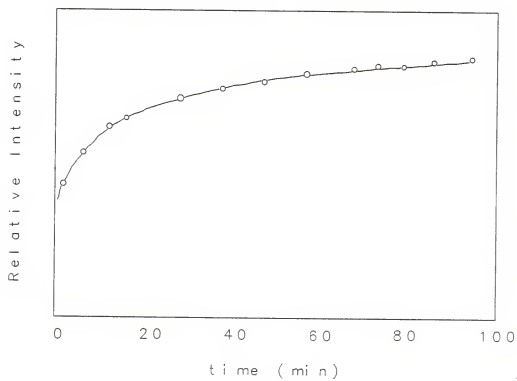
PAHs when SDS was added to the paper substrate in the presence of the thallium salt. These facts suggest that some kind of organization of the surfactant molecules on the substrate takes place, and that PAHs are associated in some particular way with the nonpolar tails of the surfactant.

The above results emphasize the ability of surfactants to organize molecules, even in the environment provided by the paper surface. Perhaps, the paper surface should not be considered simply as an active interface, but rather as a concentrated aqueous solution of cellulose, where phosphors, surfactants and metal ions have enough mobility to form organized assemblies and also interact with the glucopyranose units.

Influence of Moisture. The phosphorescence intensity of chloropromazine in the presence of SDS under forced drying conditions and as a function of time is shown in Figure 2-9. The intensity increased continuously, more rapidly at the beginning, but was still increasing at a rate of $0.15\% \text{ min}^{-1}$ after 1.5 hour. Somewhat slower rates were observed when the flow of nitrogen was 6 and 8 l min^{-1} , but no significant improvements were found with a flow higher than 10 l min^{-1} .

Similar behavior was observed when the sample was treated in the oven at 110°C for 5, 10, 20, 30 min. These experiments indicated that, after the less retained water has been eliminated from the paper surface, the removal of more water is a very slow and an increasingly more difficult process. Similar experiments performed with p-aminobenzoic acid, carbazole, and o-terphenyl gave curves (Fig. 2-9) which exhibited almost exactly the same shape as that observed for chloropromazine. Therefore, water seems to affect the phosphorescence of polar and non-polar compounds in similar ways.

Fig. 2-9. Influence of drying time on the phosphorescence intensity. Pieces of Whatman No. 1 paper were spotted with $2\mu\text{l}$ of 0.25 M TiNO_3 and $5\mu\text{l}$ of $25\mu\text{g/ml}$ chloropromazine hydrochloride in a 80/20 ethanol/water mixture, treated at 110°C for 10 min. and introduced into the sample compartment with the nitrogen flow at 10 l/min .

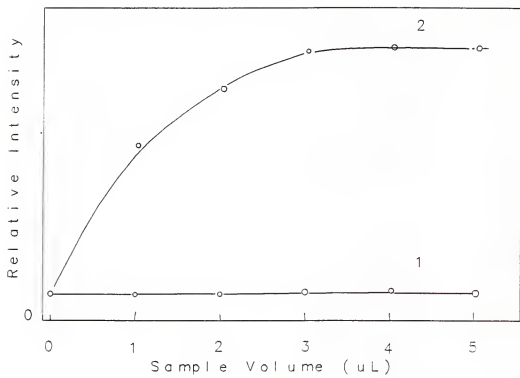


In all cases, if the stream of nitrogen was moistened by bubbling through deaerated water, the phosphorescence intensity decayed to the background level in a few seconds. However, if dry nitrogen was blown again on the surface of the paper, the phosphorescence intensity was gradually restored.

These experiments show the importance of the presence of water on RTP and suggest that attachment of the phosphors to the paper surface is a complex process, which is consistent with the swelling and entrapment mechanism proposed to explain paper-substrate RTP (81). The presence of some residual water in the paper structure seems to be unavoidable; therefore, to obtain reproducible measurements in paper-substrate RTP it is necessary to control the time and the atmosphere where the experiments are performed (e.g., gas flow and position of the sample with respect to the gas stream).

Influence of Sample Volume. The influence of increasing volumes of analyte is shown in Figure 2-10. A constant intensity was reached with more than 3 μ l, which seems to indicate that the concentration of the analyte in the center of the spot is constant and, therefore, independent of the size of the spot. Thus, if enough sample is used (e.g., 5 μ l), the error in spotting in the volume of sample delivered and even the point of application of the spotting device tip does not have a significant influence on the phosphorescence intensity. This advantageous situation is probably restricted to particular analyte/solvent systems as it seems to be for the case of chlorpromazine in the 80/20 ethanol/water mixture. The blank intensity was small and was not affected by the spot size or the presence of the surfactant. This was not true if a thallium (I) salt was

Fig. 2-10. Influence of the volume of sample: (1) blanks; (2) different volumes of a 50 $\mu\text{g/ml}$ chloropromazine solution in a 80/20 ethanol/water solution containing 3% SDS spotted on Whatman No. 1 filter paper. Five measurements on different pieces of paper were made at each point.



added, because the phosphorescence of the paper background was strongly enhanced by thallium.

Other Phenothiazine Derivatives. Spectral characteristics, enhancement factors, and limits of detection for several phenothiazine derivatives with various substituents in positions 1 (R) and 10 (R'), in the presence of SDS and both SDS and thallium (I) nitrate, are given in Table 2-8. Compounds having the same R' substituent showed similar enhancement factors. Exceptions to this rule correspond to the presence of an R group containing a hydroxyl or ester, as observed for perphenazine, thiopropazate, and fluphenazine. In these cases, higher enhancement factors were observed, either upon addition of SDS alone or SDS and thallium (I) nitrate. This is remarkable behavior because the differences are due to a change introduced several carbon atoms away from the aromatic ring system, which usually has very little influence on the phosphorescence spectra and intensities. The hydrogen-bonding capacity of the hydroxyl or ester groups can provide an additional strong anchor point between the molecule and the paper surface, and thus more rigidity can be achieved. However, it is interesting to observe that this advantage is enhanced by the presence of SDS and SDS with thallium ions. In addition, vibrational motion in the nonpolar hydrocarbon chain of the R substituent may also be hindered by the surfactant. Interaction between the anionic surfactant head and the cationic ammonium groups of the phenothiazine derivatives may also occur.

No phosphorescence was obtained in the absence or presence of SDS and thallium ions for acetophenazine maleate. Low temperature phosphorescence was also not observed for this compound (91). Acetophenazine has a methylketone group at R' and the same substituent as

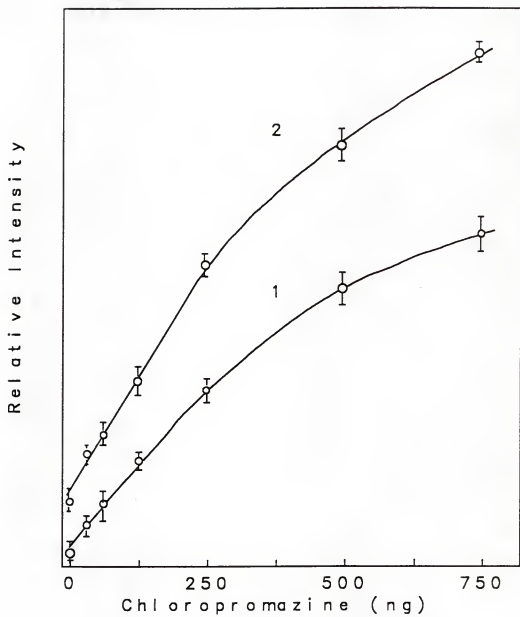
Table 2-8. Spectrum Maxima, Enhancement Factors and Limits of Detection for PS-RTP of Several Phenothiazine Derivatives in the Presence of TiNO_3 and SDS^1 .

Compound	λ_{ex} (nm)	λ_{ex} (nm)	Enhancement Factor SDS	Enhancement Factor SDS/TiNO_3	None	LOD (ng) SDS	SDS/TiNO_3
Promazine HCl		<u>258</u> , 306	1.6	3.8	0.2	0.5	0.3
Trimeprazine Tartrate		<u>258</u> , 304(sh)	1.4	3.1	0.4	0.2	0.3
Thiopropazate		<u>258</u> , 300(sh)	2.6	3.9	4.0	0.8	1.8
Perphenazine		<u>258</u> , 299	6.2	5.3	1.9	0.3	1.3
Chloropromazine HCl		<u>260</u> , 313	3.3	5.7	1.6	0.6	1.6
Prochloro- perazine edisylate		<u>257</u> , 305(sh)	3.1	4.9	7.0	0.8	1.4
Trifluopro- mazine, HCl		<u>259</u> , 303(sh)	1.5	6.7	9.5	2.0	0.6
Trifluo- perazine (HCL) ₂		<u>262</u> , 307(sh)	2.1	6.8	20.0	1.7	1.2
Fluphenazine HCl		<u>259</u> , 305(sh)	2.2	9.9	7.0	2.0	2.6
Thioridazine HCl		<u>265</u> , 310(sh)	1.6	4.3	4.7	0.8	0.6
Serentil		<u>263</u> , 307(sh)	2.2	4.2	17.0	0.6	1.1

perphenazine or fluphenazine at R. Therefore, the lack of phosphorescence could be due to the presence of the carbonyl. However, PS-RTP has been observed for other compounds with a methylketone group directly bound to the aromatic ring system, as acetophenone (1), which indicates that the particular position of the carbonyl or the possible carbonyl-hydroxyl intramolecular hydrogen bonding could account for the absence of phosphorescence. The structures of the phenothiazine derivatives used in this study are given in Appendix A.

Calibration curves and analytical figures of merit. Calibration curves for chloropromazine in the presence of SDS, with and without thallium ions, are shown in Figure 2-11. The calibration curves are approximately linear from the detection limit to 250 ng of analyte. The addition of thallium ions results in increased sensitivity; however as observed in Table 2-8, for most of the derivatives, it can also lead to poorer limits of detection. This is probably because of the enhancement of the background phosphorescence of the paper. Relative standard deviations (RSD) were obtained for chloropromazine under the conditions in Table 2-8, in the presence and absence of thallium ions using ten different pieces of paper for each case. The RSD was 6.7% with and without thallium ions. Similarly, two series of ten independent measurements of the blank were done. Limits of detection for chloropromazine were 7 ng and 4 ng, the better corresponding to the absence of thallium ions.

Fig. 2-11. Calibration curves for chlorpromazine hydrochloride: (1) 3% SDS; (2) 3% SDS and 0.1 M TiNO_3 . Both with Whatman No. 1 filter paper.



CHAPTER 3

THE EFFECT OF MAGNETITE PARTICLES ENCAPSULATED IN DIHEXADECYL PHOSPHATE (DHP) VESICLES ON THE LUMINESCENCE CHARACTERISTICS OF MOLECULES

The ability of membranes to solubilize reactants, to alter their microenvironments, and hence to affect their product distributions, stereochemistries, dissociation constants, redox potentials, and reactivities has been exploited in a variety of analytical applications. Surfactants have been found to be useful for solubilizing analytical reagents, for enhancing given analytical reactions needed for quantitative determinations, for increasing sensitivities, stabilities, and selectivities. Existing methods have been improved and entirely new ones have been developed for ultraviolet-visible (UV-VIS) absorption and luminescence, atomic emission and absorption spectroscopies, and electrochemical determinations. Observations of phosphorescence and delayed fluorescence in aqueous micellar solutions at room temperature are known (31,92). Up to now, most of the analytical work involving room temperature phosphorescence and delayed fluorescence has utilized aqueous micelles (57,58).

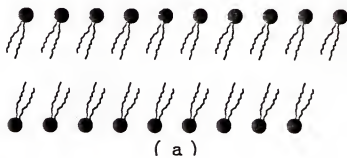
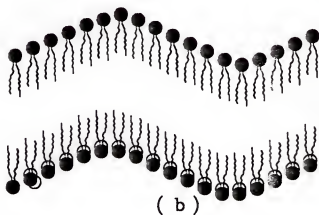
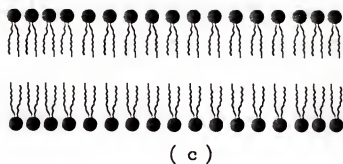
Although there are many similarities in properties between micelles and surfactant vesicles, there are also many notable differences. Surfactant vesicles are much larger entities, more static (i.e., less fluid, "more rigid") aggregates. Vesicle aggregates, once formed, cannot be destroyed by dilution, whereas micelles can. Most synthetic surfactant

vesicle systems also exhibit temperature dependent phase transition behavior in contrast to the micelle systems (31,93).

Surfactant vesicles have very similar properties to lipids. The phase transition temperature is an important property in both membranes and surfactant vesicles. Below the phase transition temperature, lipids are tilted and highly ordered. They are in their solid or "gel" state. Increasing the temperature leads to a pretransition, characterized by periodic undulations and straightening of the hydrocarbon chain. Further increase of the temperature causes the main phase transition. At temperatures above the main phase transition temperature lipids are fluid or "liquid crystalline". Figure 3-1 illustrates the effect of increasing temperature on the behavior of lipids. Most membranes are osmotically active. They shrink if electrolytes are added externally. They swell if placed in a solution which is more dilute than their internal electrolyte concentrations (94).

Vesicles are smectic mesophases of surfactants containing water between their bilayers (94,95). Prepared by sonication of surfactants such as dioctadecyldimethylammonium bromide (DODAB) or dihexadecylphosphate (DHP), they are single bilayer spherical aggregates with diameters of 300-10000 Å and bilayer thickness of about 50 Å. They can undergo a phase transition, fuse and are osmotically active. Vesicles are capable of organizing a large number of molecules in their compartments. Hydrophobic molecules can be distributed among the hydrocarbon bilayers of the vesicles. Polar molecules may move about relatively freely in vesicle-entrapped water pools, particularly if they are electrostatically repelled from the inner surface. Small charged ions can be electrostatically attached to the oppositely charged vesicle

Fig. 3-1. The phase diagram for the structures of lipids and surfactant vesicles at various temperatures: (a) at low temperatures, the lipids are arranged in tilted one-dimensional lattices; (b) at the pretransition temperature, two-dimensional arrangements are formed with periodic undulations; (c) above the main phase transition temperature, lipids and vesicles revert to one-dimensional lattice arrangements, separated from each other, and assume mobile liquid-like conformations.



Increasing Temperature

surfaces. Species having charges identical with those of the vesicles, can be anchored onto the vesicle surface by a long hydrocarbon tail (94).

The Effect of a Magnetic Field on a Molecule

The effect of an external magnetic field on photophysical properties has been studied, mainly using molecular crystals as examples. Magnetic fields have been found to affect such processes as photoconductivity, slow fluorescence caused by the annihilation of triplet excitons, photochemical oxidation, and a number of other processes in molecular crystals. Successful observations of the effect of magnetic fields on photophysical and photochemical processes in the solid phase have stimulated the search for analogous phenomena in liquids and in gases. Quenching of slow (annihilation) fluorescence by a magnetic field has been observed for a number of aromatic hydrocarbons (96-101). It has been found that when account is taken of spin-orbital interaction (SOI), which mixes singlet (S), and triplet (T) states and induces intersystem crossing (ISC), an external magnetic field causes additional mixing of the S and T states and can enhance the ISC process. This leads to the quenching of fluorescence in an isolated molecule by a magnetic field (96,97).

Herve, Nome, and Fendler reported the first observation of a substantial magnetic effect in the absence of an applied magnetic field; dramatic alteration of the photochemistry of benzophenone was observed in a surfactant vesicle in which colloidal magnetite was incorporated (102).

Most of the previous work involving surfactant vesicles has been employed as models for the study of biological transport and membrane-solute interactions (93). Surfactant vesicles have also been used in drug encapsulation studies, photochemical energy storage and conversion studies, electron transfer and charge separation studies and catalysis

(92). Here, magnetite is incorporated into DHP vesicles in an attempt to influence the luminescence properties of several molecules.

Experimental

Apparatus. Luminescence measurements were made with a Perkin-Elmer LS-5 Luminescence Spectrometer. A 3600 data station interfaced to the spectrometer was used to obtain the spectra. Excitation and emission spectral bandwidths were set at 10 nm and 5 nm, and the delay and gate times used were 0.03 ms and 9 ms, respectively for both phosphorescence and delayed fluorescence measurements; for fluorescence measurements both the excitation and emission slits were set at 3 nm. The wavelength accuracy of the LS-5 is ± 2 nm and the wavelength repeatability is ± 1 nm. A 305 nm cut-off filter was used in the emission-side filter holder, except in the measurement of 1-pyrenebutyric acid (PBA) and 1-pyrene-carboxaldehyde (PCHO), where a 360 nm cut-off filter was used instead.

Chemicals. Dihexadecylphosphate (DHP), magnetite, (Fe_3O_4), carbazole, 1-pyrenecarboxaldehyde (PCHO), 1-pyrenebutyric acid (PBA), 2-aminonaphthalene (2-AN), 7,8-benzoflavone (BF) (α -naphthoflavone), thallium(I) nitrate, anhydrous sodium sulfite, hydrochloric acid, chloroform were used as received. A 0.25 M thallium(I)nitrate stock solution was prepared; a 0.1 M solution of sodium sulfite, Na_2SO_3 was prepared daily in previously degassed water. Stock solutions of DHP (10 mg/ml), carbazole, PCHO, PBA, BF, and AN were prepared in chloroform. A stock solution 0.01 M phosphate buffer (pH 7.4) was prepared. The sources of these chemicals are given in Table 2-1.

Preparation of molecules encapsulated in DHP vesicles. Vesicles were prepared by adding 2.5 ml of the DHP stock solution to a 10 ml boiling flask; 0.5 ml of the analyte of the appropriate concentration was then added to the flask. The solvent was removed by rotary evaporation at room temperature (under vacuum). The resulting surfactant vesicle film was hydrated by the addition of 6 ml of the buffer and 0.3 ml TiNO_3 (0.25 M) which had been previously heated to between 80-90°C. The resulting suspension (containing multilamellar structures) was then returned to the rotary evaporator (no vacuum used at this point) where it was hydrated for 20 min in a water bath at 75-85°C. The suspension was then sonicated with a Sonication Cell Disruptor (Model W 185F, Heat Systems, Long Island, New York), setting 5 for 5 min at 80°C; a bluish optically transparent solution was obtained. (Sonication was limited to 5 min to minimize peroxide radical formation and buildup which could destroy the vesicles). The solution was then transferred to a Safety-Head Centrifuge (Clay Adams, Division of Becton Dickinson & Co., Parsippany, New Jersey) for 10 min, setting 4, in order to remove titanium particles from the titanium probe, and where appropriate unencapsulated magnetite particles.

After centrifuging, the opaque-blue solution was transferred to a 10 ml volumetric flask. If measurements were not performed immediately, the solutions were stored at 4°C in order to slow down fusion of the vesicles. Just prior to taking measurements, 1 ml of 0.1 M sulfite solution and enough phosphate buffer to mark were added. Luminescence measurements were taken within a 2-5 min period at room temperature.

Magnetite containing vesicles were prepared in a similar manner, with the magnetite particles (3 mg) added to the solution prior to rotary evaporation. Concentrations of magnetite encapsulated in the vesicles

were determined by atomic absorption using a Hitachi 80-80 Polarized Zeeman Atomic Absorption Spectrophotometer, after dissolving the magnetite containing vesicles in 3 M HCl.

Results and Discussion

Carbazole. Carbazole was chosen as the model compound in these studies. After addition of the sodium sulfite (61), the change in phosphorescence intensity was monitored over a period of 90 minutes in order to determine the optimum time after addition of the sulfite for measurement. From Figure 3-2, it may be seen that the intensity of the sample varied very little from the start of measurement to 90 min later, where the intensity had increased by about 6%. Calibration curves of carbazole in the DHP vesicles were prepared both in the absence and presence of magnetite particles. Two wavelength combinations were used for the calibration curves, i.e., 297/440 nm, and 330/440 nm (in the absence and presence of magnetite particles). At both sets of wavelengths, the calibration curves were found to be linear over 2.5 order of magnitude. At 297/440 nm, the limits of detection were found to be 30 ng/ml and 9 ng/ml, in the absence and presence of magnetite, respectively. At 330/440 nm, the limits of detection were found to be 30 ng/ml both in the absence and presence of magnetite. The calibration curves at 297/440 nm are shown in Figure 3-3. The precision of the method ranged from ~1% to 40%.

The characteristics of carbazole luminescence with and without magnetite are given in Table 3-1. The most interesting results observed were when the highest concentration of carbazole was used (i.e., ~50 $\mu\text{g/ml}$). First, in the emission spectrum of carbazole (no magnetite present), a peak was observed at 394 nm. This peak is not present in the

Table 3-1. Analytical Figures of Merit of Carbazole in DHP Vesicles

Analytical Figure of Merit	Without Fe_3O_4	With Fe_3O_4
Limit of Detection	30 ng/ml ^a 30 ng/ml ^b	9 ng/ml ^a 30 ng/ml ^b
Linear Dynamic Range	2.5 orders	2.5 orders
Lifetime (ns)	0.26-0.28 ^c	0.29-0.32 ^d
a. LOD obtained at 297/440 nm		0.19 ^e
b. LOD obtained at 330/440 nm		
c. Lifetimes measured in DHP vesicles at all concentrations (0.01 $\mu\text{g}/\text{ml}$ - 50.3 $\mu\text{g}/\text{ml}$) in absence of magnetite particles.		
d. Lifetimes measured in DHP vesicles at low concentrations in presence of magnetite particles (0.01 $\mu\text{g}/\text{ml}$ - 10.1 $\mu\text{g}/\text{ml}$).		
e. Lifetimes measured in DHP vesicles at highest concentration used (i.e. ~50 $\mu\text{g}/\text{ml}$) in presence of magnetite particles		

Fig. 3-2. Variation of phosphorescence intensity of carbazole with time after the addition of SO_3^{2-} ($\lambda_{\text{ex/em}} = 297/440 \text{ nm}$).

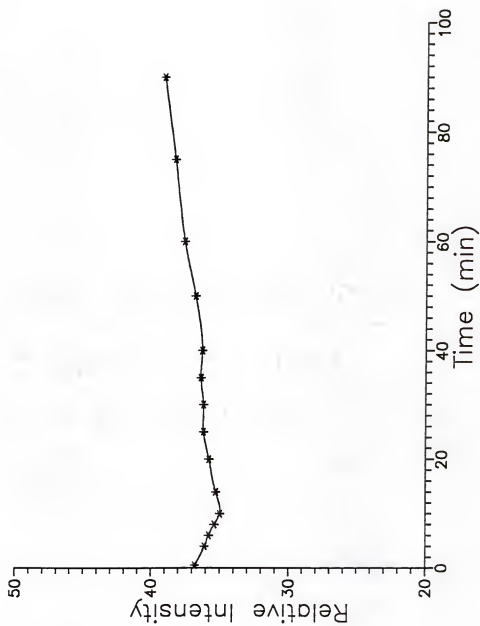
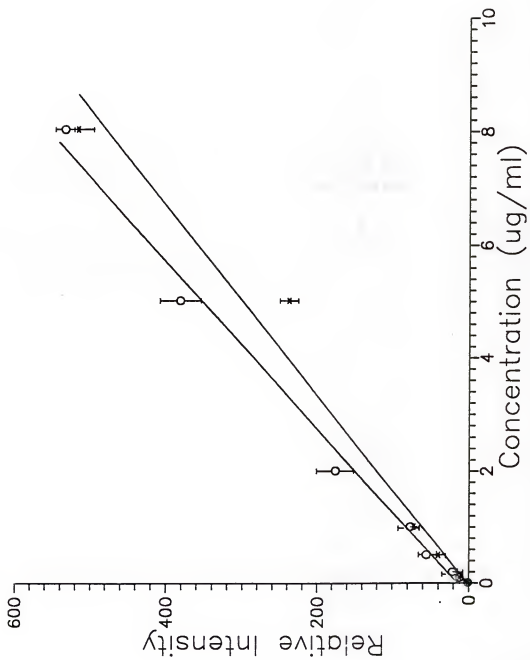


Fig. 3-3. Calibration curve of carbazole in DHP vesicles in o: presence of magnetite particles; *: absence of magnetite particles (highest concentrations omitted from graphs). ($\lambda_{ex}/nm = 297/440$ nm).



emission spectrum at any other concentration (with or without magnetite). The appearance of the peak seems to be due to a concentration effect. Secondly, there was a reversal of peak intensities between the 416 nm and 440 nm peak. At all lower concentrations (with or without magnetite), the 440 nm peak was more intense than the 416 nm peak. The contrast between the two spectra is shown in Figure 3-4. At the same concentration (~50 $\mu\text{g/ml}$), there were also differences observed in the excitation spectrum (when no magnetite was present). There were peak (intensities) reversals between the 297 nm and 330 nm peaks. A 10 nm (892 cm^{-1}) shift from 330 nm to 340 nm was observed; a slight but perceptible shift from 297 nm to 300 nm was also observed. Figure 3-5 compares the two spectra.

The lifetimes of carbazole in DHP vesicles at both low and high concentrations in the absence of magnetite particles were found to be between 0.26 - 0.28 ms. At lower carbazole concentrations in the presence of magnetite, the lifetimes were found to be quite similar, ranging from 0.29 - 0.32 ms. However, at the highest carbazole concentration used (~50 $\mu\text{g/ml}$), the lifetime of carbazole was found to decrease to 0.19 ms. This decrease in lifetime by a factor of about two-thirds seems to be the result of more efficient intersystem crossing caused by additional mixing of the singlet (S) and triplet (T) states.

The nonlinearity in the signal-concentration relationship observed at higher concentrations may be a result of one or more processes. First, at the highest concentrations, a small but perceptible shift was observed in the excitation maxima (297 nm to 300 nm), and peak reversals in the emission spectrum (416 nm and 440 nm) peaks were seen. Secondly, quenching of the signal may occur because of triplet-triplet annihilation (TTA). Thirdly, the inner filter effect may also be contributing to the

Fig. 3-4. Emission spectra of carbazole in DHP vesicles. Curve (a) in absence of magnetite particles; curve (b) in presence of magnetite particles. $\lambda_{ex} = 297$ nm (figures not on the same scale; offset for clarity).

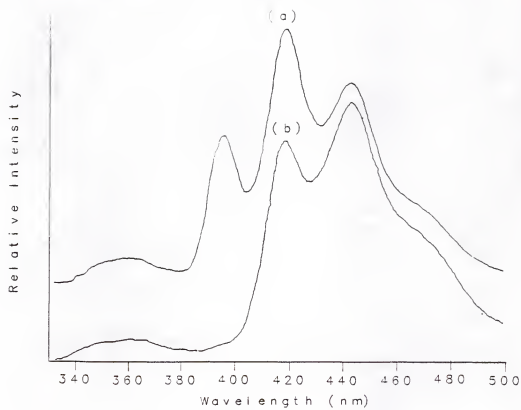
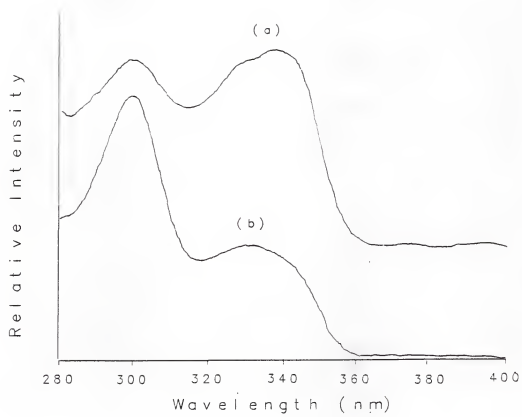


Fig. 3-5. Excitation spectra of carbazole in DHP vesicles. Curve (a) in absence of magnetite particles; curve (b) in presence of magnetite particles. $\lambda_{em} = 440$ nm (figures not on the same scale; offset for clarity).



curvature observed at high concentrations (57,103). The average concentration of magnetite found in the vesicles was 3.6×10^{-8} M.

Because of the varied results obtained at low and high concentrations of carbazole, the luminescence characteristics of other molecules with various functional groups were investigated both at low and high concentrations in the absence and presence of magnetite. Table 3-2 gives the luminescence characteristics of the various molecules in the presence and absence of magnetite.

PBA. Weak phosphorescence was observed for PBA. The peaks at 377 nm and 396 nm may be attributed to delayed fluorescence; similar peaks are seen in the parent compound, pyrene. The peak at ~ 696 nm is partially a result of second order scatter. The lifetime of PBA was found to be ~ 1.2 ms both in the absence and presence of magnetite at low concentrations. This value is in close agreement with that obtained by Skrilec and Cline Love (58). It has been suggested that the fairly long lifetime of PBA may occur because this molecule is fairly bulky, and therefore, because of its lower mobility, it may spend a longer time within the protective environment of the vesicle (58). At higher concentrations of PBA, the lifetimes were found to be 0.79 ms and 0.88 ms in the absence and presence of magnetite, respectively. The shorter lifetimes may occur because of quenching or triplet-triplet annihilation. Although Cline Love reported intense phosphorescence for this molecule, weak phosphorescence was observed in this case. This may be a result of two factors. First, because the vesicle was prepared in phosphate buffer (pH = 7.4), some degree of ionization probably occurred in the PBA molecule, hence affecting the quantum yield. There may also have been some repulsion between the negative charge of the carboxyl group and the

Table 3-2. Effect of Magnetite on the Luminescence Characteristics of Several Molecules

Molecule Concentration ($\mu\text{g}/\text{ml}$)	PBA	7, 8 BenzoFlavone	PCHO	2-Aminonaphthalene
		% change in Phosphorescence ^a		
~5	0	-25%	N/D	N/D
~50	0	-33%	N/D	N/D
		% change in Delayed Fluorescence ^a		
~5	N/D ^b	N/D	+42%	-5%
~50	N/D	N/D	-29%	+15%
		% change in Fluorescence ^a		
~5	N/D	-5%	N/D	N/D
~50	N/D	-17%	-19%	N/D
		Lifetimes		
~5	1.19 ^c , 1.12 ^d	N/D	N/D	N/D
~50	0.79 ^c , 0.88 ^d	N/D	N/D	N/D

a. Percent change in luminescence = $[\text{lum}(\text{no Fe}_3\text{O}_4) - \text{lum}(\text{Fe}_3\text{O}_4)] / \text{lum}(\text{no Fe}_3\text{O}_4) \times 100$;

b. N/D = not determined

c. Lifetime determined in the absence of Fe_3O_4

d. Lifetime determined in the presence of Fe_3O_4

negative charge of the anionic DHP vesicle. No change in the phosphorescence intensities of PBA in the presence and absence of magnetite at both low and high concentrations was observed.

BF. BF gave both fluorescence and phosphorescence in the vesicle solution. In nonpolar solvents, such as heptane, fluorescence is not observed from this molecule. The observation of fluorescence in the vesicle suggests that 7,8-benzoflavone is probably located in the more polar part of the vesicle. In this case, the lowest excited state is $\pi \rightarrow \pi^*$ whereas in nonpolar solvents, the lowest excited state is $n \rightarrow \pi^*$. In nonpolar solvents, intersystem crossing is favored (104). Because this molecule seems to be located in the more polar region of the vesicle, this more polar environment of the vesicle may be contributing to a decrease in the phosphorescence intensity observed (i.e., a lower rate of ISC).

It was also observed that at low concentrations, the relative phosphorescence intensities were higher than those of higher concentration solutions. This observation of reduced phosphorescence intensities may be due to quenching or triplet-triplet annihilation or both processes occurring simultaneously. It should also be noted that in the presence of magnetite, the percentage of phosphorescence quenching at low and high concentrations are 25% and 33%, respectively. A similar trend was observed for the fluorescence intensities (i.e. higher relative intensities at lower concentrations). The percentage quenching at low and high concentrations in the presence of magnetite was found to be 5% and 17%, respectively.

PCHO. No phosphorescence was observed for this molecule. Similar to the other molecules used in this study, higher relative fluorescence and delayed fluorescence intensities were seen at low concentrations

rather than at the higher concentrations where one or more type of quenching mechanisms may be occurring. At low concentrations, in the presence of magnetite particles, the delayed fluorescence intensity increased 42%, whereas at high concentrations a reduction in relative intensity of 29% was observed. The increase in delayed fluorescence at low concentrations may be explained by more efficient intersystem crossing, whereas at high concentrations even though intersystem may be more efficient, quenching mechanisms may be more predominant.

AN. This compound was very highly fluorescent at all concentrations used (direct fluorescence of the molecule could not be observed due to saturation of the photomultiplier tube). There was very little change in the delayed fluorescence intensity both at low and high concentrations in the presence or absence of magnetite; the amino group seemed to be little affected by the presence of magnetite.

Conclusions

Although the inclusion of magnetite particles into the vesicles did not prove to be as analytically useful as hoped, vesicles could conceivably be more interesting systems than micelles for luminescence studies. The precision of the method ranged from ~1% to ~40%. With the judicious manipulation of the conditions in which vesicle solutions are prepared, change of luminescence properties of many molecules may be achieved. If there were a way of including more magnetite particles in the vesicles, more dramatic changes in luminescence might be achieved.

CHAPTER 4

THE EFFECT OF BROMINATED ALCOHOLS ON THE LUMINESCENCE PROPERTIES OF MOLECULES INCLUDED IN CYCLODEXTRINS

Introduction

Cyclodextrins (CDs), (also called Schardinger dextrins, cycloamyloses, cycloglucans) are a series of oligosaccharides produced by the action of the amylase of *Bacillus macerans* on starch and related compounds. Cyclodextrins are composed of α -(1,4)-linkages of a number of D(+)-glucopyranose units (62). They consist of six, seven, or eight glucose monomers arranged in a doughnut shaped ring, which are denoted α -, β -, and γ -, respectively. The specific coupling of the glucose monomers gives the cyclodextrins a rigid, conical molecular structure with a hollow interior of a specific volume. The internal cavity is hydrophobic in nature. Cyclodextrins have the ability to complex a variety of "guest" molecules, such as aromatics, halides, hydrogen halides, fatty acids and their esters. To form an inclusion complex, the guests must satisfy the size criterion of fitting at least partially into the internal cavity of the cyclodextrin (105).

The formation of inclusion complexes is due to several forces acting simultaneously. The extent to which each of them is involved is related to the substrate concerned. Some of these forces are van der Waals, the formation of hydrogen bonds between the guest and the OH groups of the cyclodextrin, and hydrophobic interactions. On inclusion, the guest molecule must expel the water molecules already present and strip off its

own hydration sphere. It has been shown that the predominant forces are van der Waals and hydrophobic interactions. The inherent ring structure of the cyclodextrins is, however, decisive as inclusion complexes are only formed if there is a good spatial fit between the guest and host components (105). The internal diameters of α -CD, β -CD, and γ -CD have been found to be ~ 5.7 Å, ~ 7.8 Å, and ~ 9.5 Å, respectively. The height of the cavity of all three cyclodextrins is ~ 7 Å.

Cyclodextrins have been used in many areas of science. They have been used as enzyme models, to catalyze chemical reactions, and in the production of pharmaceuticals, pesticides, and foodstuffs where the microencapsulated active and aromatic substances enclosed within them are protected from the effects of light and atmosphere and can be easily handled and stored in powder form. Substances which are not very soluble in water become more soluble in the presence of cyclodextrins bound to a polymer carrier and have already been employed in gel inclusion and affinity chromatography (105,106).

While cyclodextrins have been used in several areas of chemistry, they have also been used to enhance many types of luminescence spectroscopy. Cyclodextrins have been found to enhance the chemiluminescence of the reaction between hydrogen peroxide and 10,10'-dimethyl-9,9'-biacridinium nitrate. Up to a 20-fold increase in chemiluminescence intensity was observed in 10^{-2} M β -CD. The enhancement of the chemiluminescence intensity was attributed to an increase in the excitation efficiency and the rate of the reaction through the inclusion of a reaction intermediate in the cyclodextrin cavity (107). Cyclodextrins were also found to enhance the chemiluminescence intensity by factors of up to 300 of aqueous peroxyoxalate chemiluminescence

resulting from the reaction of 4,4'-[oxyallybis[(trifluoromethylsulfonyl)-imino]ethylene]bis[4-methylmorpholinium trifluoromethane sulfonate] with hydrogen peroxide (108).

However, the majority of the luminescence studies in cyclodextrins have involved fluorescence and to a lesser extent, phosphorescence. The enhancement of fluorescence and the observation of phosphorescence may be attributed to the compartmentalization and shielding of the excited singlet and triplet species from nonradiative decay processes that can occur in bulk solution (63, 109). Many studies have involved binary complexes (1:1) between cyclodextrin and fluorophore (110-113). More recently, ternary (1:1:1) inclusion complexes have been studied (114-118).

There have been several studies of various types of ternary inclusion complexes. Among them have been cyclodextrin-fluorophore complexes with surfactants (119-121). It was found that when the surfactants were used below their critical micelle concentration (c.m.c.) with the cyclodextrins, a more hydrophobic environment was created. The more hydrophobic environment was due to the replacement of water within the cavity by the molecule and surfactant. Above the c.m.c., micelles of the surfactants were formed and the guest molecule was transferred from the cavity of cyclodextrin to the micelle (119-121).

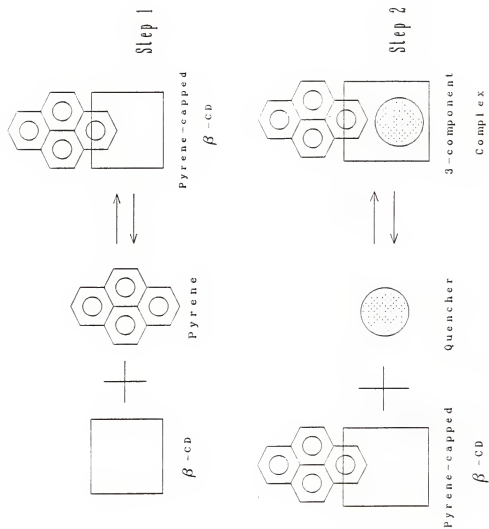
The observation of exciplex formation was also noted under certain experimental conditions (116, 122). Hamai (116) observed exciplex formation for both pyrene and 1-pyrenesulfonate when included in the cavity of β -CD when aniline was added in concentrations of above 1×10^{-2} M. Both pyrene and 1-pyrenesulfonate are too bulky to be included completely in the β -CD cavity; because of steric hindrance, an additional molecule can be incorporated in the void interior of the cavity. The possible

mechanisms for this process are illustrated in figure 4-1. The observation of exciplex emissions in β -CD was also reported by Kano et al. (122). In this study, exciplex formation was observed from acenaphthene and triethylamine (TMA) at β -CD of 1×10^{-2} M. Weaker or no exciplex emission was observed at lower concentrations of β -CD used in this study. At lower β -CD concentrations, fluorescence quenching of the guest molecules was observed under similar experimental conditions (122).

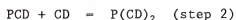
However, many other studies of ternary inclusion compounds have focused on the effect of alcohols as the third component, and on the inclusion and spectrophotometric properties of these complexes. Ueno et al. found that cyclohexanol acted as a space-regulator in the α -naphthylloxyacetic acid- γ -CD system (123). When α -naphthylloxyacetic acid was included in γ -CD in the absence of cyclohexanol, little enhancement in the fluorescence of this molecule was observed; however, when cyclohexanol was present, a marked enhancement in the fluorescence signal was observed. A marked depression in the fluorescence signal was observed when β -CD was used with cyclohexanol instead of γ -CD. The cavity of γ -CD has been found to be too large to fit the derivatives of benzene and naphthalene (123). It was suggested that in γ -CD, the fluorophore is sandwiched between the hydrophobic wall of the γ -CD and cyclohexanol; the environment of α -naphthylloxyacetic acid in this system is found to be similar to that observed in β -CD in the absence of cyclohexanol. This study illustrated the effect the different cavity sizes of CDs can have on the luminescence of molecules in the presence of a third component.

Alcohols have also been found to accelerate the inclusion process and in many cases can cause further association compounds. Nakajima (124) found that the rate of inclusion of pyrene in β -CD was enhanced in the

Fig. 4-1 Stepwise formation of a three-component complex in a cyclodextrin.



presence of ethanol. He also discovered that further association of the pyrene-ethanol- β -CD complex with another molecule of β -CD was accelerated by the addition of ethanol. He proposed the following scheme for his observation:



where P is pyrene, CD is cyclodextrin and PCD the inclusion complex between pyrene and cyclodextrin, $P(CD)_2$ the 1:2 complex between pyrene and cyclodextrin.

Alcohols also have been found to affect significantly the lifetimes of molecules included in the cyclodextrin cavities. Nelson et al. (125) investigated the effect of alcohols of various lengths on the lifetimes of pyrene included in γ -CD. It was found that the lifetime of pyrene in the cyclodextrin increased with increasing alcohol chain length. They attributed this observation to the interaction of the alcohols with the pyrene- γ -CD complex in a manner that further shielded the pyrene from contact with the bulk aqueous environment. Alcohols also have been found to enhance formation constants of molecules included in cyclodextrins by one or two orders of magnitude, compared to the formation constants obtained in the absence of alcohols (126).

Room temperature phosphorescence (RTP) has been observed in cyclodextrins, usually in the presence of the heavy atom reagent, 1,2-dibromoethane (DBE) (64,65). However, even though this heavy atom reagent has been introduced as a third component to promote the excited triplet formation in phosphorescence studies, this component is not thought to be an active participant in determining the complexation properties of the cyclodextrin molecule (109). In my study, the effect of brominated

alcohols on the luminescence properties of several molecules will be studied.

Although DBE produces very intense phosphorescence from some molecules in cyclodextrins, there are two distinct drawbacks associated with this compound. First, the EPA has warned that this molecule is potentially very carcinogenic. Secondly, because it is very water insoluble, the solutions where DBE has been used were very cloudy (64,65). Brominated alcohols are somewhat more water soluble and may provide an alternate method for inducing RTP in fluid solutions.

Experimental

Reagents. α -, β -, γ -cyclodextrin, 2-bromoethanol (BE), 2,3-dibromopropanol (BP), 1,2-dibromoethane (DBE), 2-propanol, methanol, acetone, ethanol, 1-naphthol, acenaphthene, naphthalene, pyrene, phenanthrene, phenazine, phenanthridine, 1-bromonaphthalene, 2-bromonaphthalene, lepidine, isoquinoline, fluorene, fluoranthrene, 9-nitroanthracene, 2-nitrofluorene, 1-nitropyrene, sodium sulfite were used as received. The sources of these chemicals are given in Table 2-1.

Apparatus. Luminescence measurements were made with a SPEX Fluorolog 2+2 spectrofluorometer (SPEX Industries, Metuchen, NJ). The instrument is equipped with a single excitation monochromator with a spectral band-pass of 3.6 nm/mm and with a double emission monochromator with a spectral band-pass of 1.8 nm/mm. The instrument acquires data in the photon counting mode. Spectra were stored on the hard disk of a PC which was interfaced to the spectrofluorometer. No time discrimination was used to distinguish between fluorescence and phosphorescence. Spectra were not corrected for instrumental response.

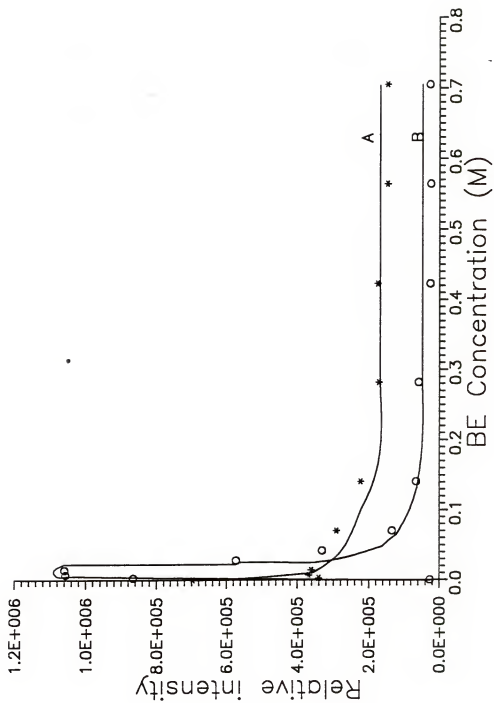
Procedure. Solutions of α -, β -, and γ -cyclodextrins (1×10^{-2} M), and sodium sulfite were prepared in nanopure water which had been previously degassed with nitrogen. Stock solutions of the lumiphors were made in acetone or methanol. Serial dilutions were made where appropriate.

Preparation of Inclusion Compound. Between 50 μ l and 200 μ l aliquots (usually 100 μ l) of the lumiphor at the appropriate concentration was added to a 5 or 10 ml volumetric flask (which had been previously acid washed and then rinsed with methanol and oven dried). The volumetric flasks were then placed on a hot plate where the solvent was very gently evaporated. Appropriate amounts of the brominated alcohols, or nonbrominated alcohols, where appropriate, were added. 0.5 ml or 1.0 ml of the appropriate cyclodextrin stock solution was added to a 5 ml or 10 ml volumetric flask, respectively, to make a final concentration of 1×10^{-3} M. 1 ml of sodium sulfite was added and water was added to the mark. The volumetric flasks were shaken vigorously and then put into an ultrasonic bath (Cole Palmer, Chicago, IL) for 30 minutes. Luminescence measurements were taken shortly thereafter.

Results and Discussion

2-Bromoethanol (BE). The effect of increasing BE concentration on the fluorescence and phosphorescence of acenaphthene was investigated. The results are shown in Figure 4-2. Two conclusions can be made; first, upon increasing BE concentration, the fluorescence signal is quenched, followed by a gradual levelling off (curve a), and secondly, there is a very rapid increase in the phosphorescence intensity, followed by a precipitous decrease in the signal, followed by a levelling off of the signal (curve b). There are two possible reasons for the observed behavior. First, CDs can form complexes with alcohols. Not only are 1:1

Fig. 4-2. Effect of 2-bromoethanol concentration on: Fluorescence (curve a) and Phosphorescence (curve b) of acenaphthene (1×10^{-5} M) in β -CD (1×10^{-3} M) and Na_2SO_3 (1×10^{-2} M).



(alcohol: cyclodextrin) complexes possible, but higher complexes such as 2:1, and 3:1 complexes are known to exist (depending on the length of the alcohol chain). The formation of higher alcohol-cyclodextrins complexes is more likely at higher concentrations of alcohol (127). Second, at higher alcohol concentrations, the bulk properties of the aqueous solvent system are more favorable for the solvation of the included molecule, so the equilibrium could be shifted towards the uncomplexed molecule (126).

2,3-Dibromopropanol (BP). The effect of BP also was investigated on the luminescence properties of acenaphthene. Similar type behavior on the fluorescence and phosphorescence of this molecule observed with BE was also observed with BP; see Figure 4-3. However, the alcohol concentration at which maximum phosphorescence intensity was observed was less with BP (1.95×10^{-3} M) than with BE (1.4×10^{-2} M). The intensity obtained with BE was considerably higher by a factor of ~ 17 .

BP was found to induce phosphorescence from naphthalene in β -CD whereas BE did not do so under similar experimental conditions, i.e. the same concentration of naphthalene, β -CD, and alcohol. The results of increasing BP concentration on the fluorescence and phosphorescence intensities are shown in Figure 4-4. However, the concentration of BP which induced the maximum phosphorescence intensity in this case was 3.89×10^{-2} M.

These results illustrate the effect of two different alcohols on the luminescence properties of molecules of different sizes and shapes. In addition, it seems that for each alcohol-analyte-CD combination, there seems to be an optimum alcohol concentration where the maximum enhancements in signals are observed (phosphorescence in this case) (117).

Fig. 4-3. Effect of 2,3-dibromopropanol concentration on: Fluorescence (curve a) and Phosphorescence (curve b) of acenaphthene (1×10^{-5} M) in β -CD (1×10^{-3} M) and Na_2SO_3 (1×10^{-2} M)

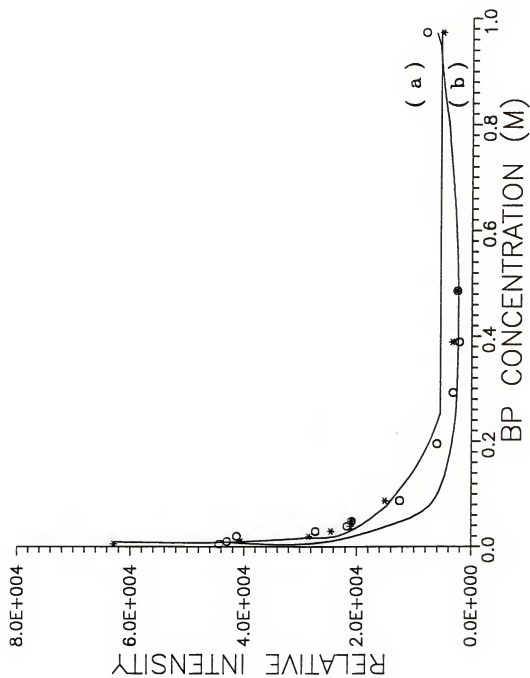
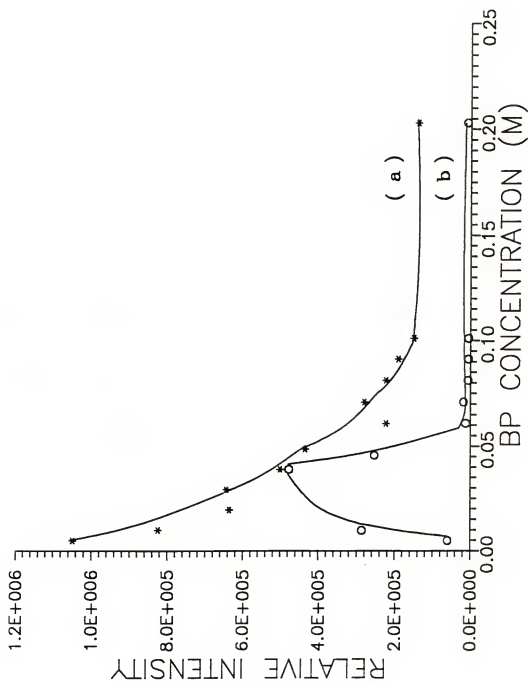


Fig. 4-4. Effect of 2,3-dibromopropanol concentration on: fluorescence curve (a) and phosphorescence (curve b) of naphthalene (1×10^{-5} M) in β -CD in β -CD (1×10^{-3} M) and Na_2SO_3 (1×10^{-2} M).



β -CD concentration. The effect of increasing β -CD concentration on the luminescence properties of acenaphthene was investigated. The results are shown in Figure 4-5. For the phosphorescence (curve b), a sharp increase in the intensity is observed, followed by a decrease and levelling off of the signal. For the fluorescence intensity, a sharp decrease in the fluorescence intensity is observed followed by a levelling off of the signal. This sharp decrease in the fluorescence signal is probably because the lumiphor is brought into closer contact with the brominated alcohol in the presence of the cyclodextrin. The fact that there was a levelling off or a decrease in the luminescence signals at higher concentrations of β -CD, may be a result of the formation of higher complexes such as 1:2 or even 2:2 lumiphor-CD complexes. This type of behavior is well documented in the literature (127,128).

Na_2SO_3 concentration. Sodium sulfite was used in this system to remove oxygen in order for phosphorescence to be observed (61). Although cyclodextrins do afford some protection from the quenching effects of oxygen, compared to other types of organized media, these systems are generally purged with nitrogen or subjected to freeze-thaw cycles in order to remove oxygen so that strong signals may be observed. Sodium sulfite provides an alternate method to remove oxygen from these systems. The effect of increasing sodium sulfite concentration on the luminescence of acenaphthene is shown in Figure 4-6. A dramatic increase in the phosphorescence intensity, followed by a levelling off of the intensity was observed. Similar behavior was observed for the fluorescence intensity.

Effect of cyclodextrins on the luminescence of position isomers.

Differences in the luminescence properties of the 1- and 2-bromo

Fig. 4-5. Effect of β -CD concentration on: fluorescence (curve a) and phosphorescence (curve b) of acenaphthene (1×10^{-5} M), BE (1.4×10^{-2} M) and Na_2SO_3 (1×10^{-2} M).

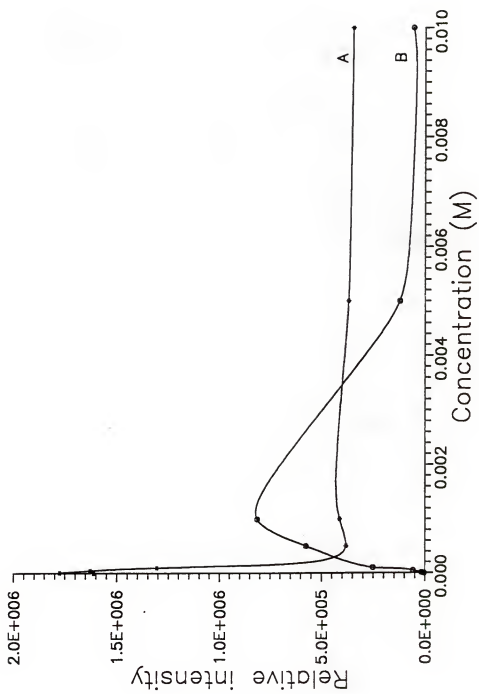
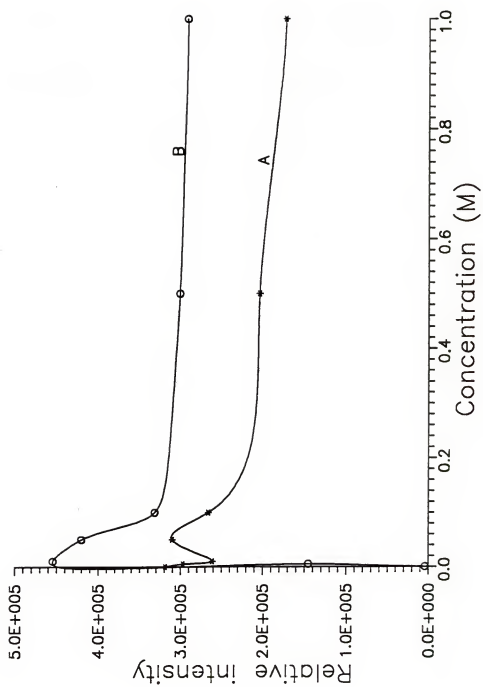


Fig. 4-6. Effect of Na_2SO_3 concentration on: fluorescence (curve a) and phosphorescence (curve b) of acenaphthene (1×10^{-5} M), BE (1.4×10^{-2} M) and β -CD (1×10^{-3} M).



derivatives of naphthalene in β -CD were observed. These two molecules are known to phosphoresce in the absence of an external heavy atom. Inclusion of 1-bromonaphthalene in β -CD produced moderate phosphorescence, whereas the inclusion of 2-bromonaphthalene in β -CD produced very weak phosphorescence. In this case, the fluorescence of this molecule was more intense. These observations illustrate the discriminatory effect of the host-guest geometries. It has been found through circular dichroism studies that 2-substituted naphthalene derivatives are included axially in the cavity of β -CD, whereas for the 1-substituted derivatives, both axial and equatorial inclusion are possible (129). In this case, it has been suggested that the bromine atom on the 1-derivative was shielded from water, whereas with the 2-derivative, the bromine atom was in contact with the bulk solution (130), hence explaining the above observations.

It was also interesting to note that upon addition of DBE, BE and BP, the phosphorescence intensities were enhanced by factors ranging from 16 to 65 for 1-bromonaphthalene. Similarly, upon the addition of DBE, BE and BP, the phosphorescence intensities were enhanced by factors ranging from 1 to 274 for 2-bromonaphthalene, although the signals of 1-bromonaphthalene in all cases were more intense than those observed for 2-bromonaphthalene.

Effect of Different Size CD Cavities With
Various Length Alcohols on the Luminescence
Properties of Different Molecules

The effect of α -, β -, and γ -CDs in the presence of various length alcohols (brominated and their nonbrominated counterparts) on the luminescence properties of four different molecules--naphthalene, acenaphthene, pyrene and phenanthrene was investigated. The concentration

of all analytes used in this study was 10^{-4} M; all CDs were 10^{-3} M and all alcohols and DBE were 1.4×10^{-2} M.

Naphthalene. For naphthalene, the three cyclodextrins were used. No enhancement in the fluorescence intensities were observed in the presence of α -CD under any conditions. No phosphorescence was also observed. This suggests that the cavity of α -CD is too small to include naphthalene, although it is known that naphthalene fits tightly into the cavity of β -CD (131). On the other hand, enhancements of the fluorescence intensities of this molecule were observed in β -CD. Phosphorescence was observed in the presence of BP and DBE. In γ -CD, no enhancement in the fluorescence signal was observed under any of the experimental conditions used; no phosphorescence was observed also. The results obtained in the presence of γ -CD were identical to those obtained when α -CD was used. These results suggest that the cavity of γ -CD is too large for naphthalene to form an inclusion compound, i.e. naphthalene has no interactions with the cavity of γ -CD (132). The environment of naphthalene in γ -CD is essentially equivalent to that experienced in bulk solution. The results are shown in Table 4-1.

Pyrene. For pyrene, only β -CD and γ -CD were used. Enhancements of the fluorescence intensities in β -CD ranged from 1.1 to 4.4 in the presence of the nonbrominated alcohols; the largest enhancements were observed when 2-propanol was used. This result indicates that of the two nonbrominated alcohols used, the more stable three-component complex of pyrene-CD was formed with 2-propanol. In the presence of the alcohols used in this study, a broad band emission was observed from pyrene in β -CD. This broad band emission is not due to excimer formation since two molecules of pyrene cannot fit into the cavity of β -CD; rather, this band

Table 4-1. Effect of Various CDs with Various Alcohols on the Luminescence of Naphthalene^d

Conditions Fluorescence (Intens)		Phosphorescence (Intens)
H ₂ O	1	N/O
<u>α-CD</u>		
no alcohol	1 ^a	N/O
w/ethanol	1 ^a	N/O
w/2-propanol	1 ^a	N/O
w/BE	0.8 ^a	N/O
w/BP	0.4 ^a	N/O
w/DBE	0.2 ^a	N/O
<u>β-CD</u>		
no alcohol	1.3 ^a	N/O
w/ethanol	1.2 ^a	N/O
w/2-propanol	1.1 ^a	N/O
w/BE	0.9 ^a	N/O
w/BP	0.6 ^a	1 ^b
w/DBE	0.04 ^a	1.8 ^b
<u>γ-CD</u>		
no alcohol	1.0 ^a	N/O
w/ethanol	1.0 ^a	N/O
w/2-propanol	1.0 ^a	N/O
w/BE	0.86 ^a	N/O
w/BP	0.4 ^a	N/O
w/DBE	0.2 ^a	N/O

a: FL Intensity compared to that in H₂O.

b: PHOS Intensity compared to that in β -CD/BP.

is attributed to exciplex formation. Pyrene is known to form complexes with alcohols above a certain concentration (133, 134). By contrast, this broad band emission was not observed with DBE. This piece of evidence further supports the contention that DBE is not an active participant in the complexation process (109).

In the cavity of γ -CD, lower enhancements in the fluorescence intensities were observed. This is because of the formation of excimer fluorescence at the expense of the monomer fluorescence. The cavity of γ -CD is known to be large enough to accommodate two molecules of pyrene. Even at concentrations of 2×10^{-6} M under the above conditions, excimer formation is observed. The results of these experiments are summarized in Table 4-2. Many of the spectra of pyrene under various conditions are shown in Appendix B.

Acenaphthene. Both fluorescence and phosphorescence of this molecule were observed under all conditions when included in β -CD. There was a slight decrease in the fluorescence signal upon inclusion in β -CD (without and with ethanol). However, there was an enhancement of the fluorescence signal when 2-propanol was used. Acenaphthene, like pyrene is a bulky molecule and fits shallowly into the cavity of β -CD. Of the alcohols used, 2-propanol seems to form the most stable ternary complex with acenaphthene- β -CD. Like pyrene in the cavity of γ -CD, excimer fluorescence was observed at the expense of monomer fluorescence. The results of these experiments are summarized in Table 4-3.

Phenanthrene. The fluorescence of phenanthrene was enhanced when included in β -CD. The enhancement factors in γ -CD were similar. However, unlike pyrene and acenaphthene, no excimer fluorescence was

Table 4-2. Effect of Various CDs with Various Alcohols on the Luminescence of Pyrene

Conditions	Fluorescence	Phosphorescence	I Exc/Mon
H ₂ O	1.0	N/O	1.7

 β -CD

no alcohol	1.1 ^a	N/O	N/O
w/ethanol	1.4 ^a	N/O	N/O ^b
w/2-propanol	4.4 ^a	N/O	N/O ^b
w/BE	1.6 ^a	N/O	N/O ^b
w/BP	0.9 ^a	N/O	N/O ^b
w/DBE	0.2 ^a	v.wk	N/O

 γ -CD

no alcohol	1.4 ^a	N/O	1.9
w/ethanol	1.2 ^a	N/O	5.4
w/2-propanol	1.3 ^a	N/O	3.8
w/BE	1.2 ^a	N/O	6.9
w/BP	0.6 ^a	N/O	13
w/DBE	0.4 ^a	N/O	8.9

a: Intensity compared to that in water.

b: Broad band observed; may be due to exciplex formation.

c: Concentration of pyrene is 1×10^{-4} M; all third components are 1.4×10^{-2} M. All [CDs] are 1×10^{-3} M.

Table 4-3. Effect of Various CDs with Various Alcohols on the Luminescence of Acenaphthene^d

Conditions	Fluorescence	Phosphorescence	I _{exc} /mon
in H ₂ O	1.0	N/O	N/O
<u>in β-CD</u>			
no alcohol	0.78 ^a	1 ^b	N/O
w/ethanol	0.28 ^a	0.22 ^{b,c}	N/O
w/2-propanol	1.23 ^a	0.24 ^{b,c}	N/O
w/BE	0.18 ^a	2.83 ^{b,c}	N/O
w/BP	0.16 ^a	0.26 ^{b,c}	N/O
w/DBE	0.42 ^a	3.45 ^{b,c}	N/O
<u>in γ-CD</u>			
no alcohol	0.69 ^a	N/O	5.9
w/ethanol	0.81	N/O	5.2
w/2-propanol	0.60	N/O	6.5
w/BE	0.53	N/O	5.3
w/BP	0.19	very weak	0.97
w/DBE	0.09	very weak	1.04

a: FL Intensity compared to that in H₂O.

b: Phosphorescence observed without heavy atom.

c: Phosphorescence intensity compared to that of molecule in β -CD.

d: Concentration of Acenaphthene is 1×10^{-4} M; all third components are 1.4×10^{-2} M. All [CDs] are 1×10^{-3} M.

Table 4-4. Effect of Various CDs with Various Alcohols on the Luminescence of Phenanthrene^d

Conditions. Fluorescence ^a		Phosphorescence ^b
H ₂ O	1.0	N/O
β -CD		
no alcohol	1.7	N/O
w/ethanol	1.2	N/O
w/2-propanol	1.7	N/O
w/BE	1.5	N/O
w/BP	1.0	N/O
w/DBE	0.6	very strong
γ -CD		
no alcohol	1.3 ^c	N/O
w/ethanol	1.8 ^c	N/O
w/2-propanol	1.5 ^c	N/O
w/BE	1.4 ^c	N/O
w/BP	0.5 ^c	N/O
w/DBE	0.4 ^c	N/O

a: Fluorescence measured at $\lambda_{\text{ex/em}}$ 285/364 nm.

b: Phosphorescence measured at $\lambda_{\text{ex/em}}$ 285/495 nm.

c: Intensity compared to that in water.

d: Concentration is 1×10^{-4} M; all third components are 1.4×10^{-2} M. All [CDs] are 1×10^{-3} M.

observed in γ -CD. The phosphorescence of phenanthrene was observed only under one condition, when DBE was present.

The fluorescence enhancements of phenanthrene were observed at 285/364 nm; however, the most intense peaks were not used to calculate the enhancement factors, because the ratio of the two most intense peaks (III/IV) changed under different experimental conditions. Therefore, the fluorescence enhancements would probably be larger, had these peaks been used. The five vibronic peaks of phenanthrene were better resolved in β -CD than in γ -CD. The five vibronic peaks I-V of phenanthrene are found at 345-6, 363-4, 383-5, 405-6, and 428-32 nm, respectively. This is probably due to the fact that phenanthrene forms a better inclusion compound in β -CD than in γ -CD. The results of these experiments are summarized in Table 4-4. Some of the spectra under various experimental conditions are shown in Appendix B.

Nitrogen Heterocycles. Several nitrogen heterocycles including quinoline, isoquinoline, phenazine, lepidine, 7,8-benzoquinoline were investigated using the brominated alcohols as the heavy atom species to induce phosphorescence. Little or no phosphorescence was observed with the compounds used in this study with either BE or BP, except in the case of lepidine where very weak phosphorescence was observed at ~594 nm. In addition, the fluorescence spectra of lepidine resembled that of the protonated species. For all the other nitrogen heterocycles studied, the emission spectrum observed was that of the unprotonated species. The reason for not observing phosphorescence from these compounds may be because the nitrogen heterocycles are more soluble than their carbocyclic analogues (65). The addition of the alcohols may be thought to shift the equilibrium to the bulk solution where only fluorescence is observed.

Other Compounds. Table 4-5 shows the effect of the three heavy atom species on the luminescence characteristics of several molecules. The concentrations of all heavy atom species are the same, 1.4×10^{-2} M. For fluorene, the most effective species was BP. For fluoranthene in γ -CD, the most effective species was BP.

The spectrum of 1-nitropyrene resembled that of its parent compound, pyrene. In γ -CD, excimer fluorescence was also observed. For 9-nitroanthracene in β -CD and γ -CD, weak to moderate signals were observed under various conditions. Weak phosphorescence was generally observed for 2-nitrofluorene in β -CD, whereas in γ -CD, even weaker signals were observed. These compounds illustrate the critical relationship between the size of the molecule, alcohol and cyclodextrin in the formation of a ternary complex.

Analytical figures of merit. Table 4-6 gives the analytical figures of merit for three compounds under various conditions. For 1-naphthol, at the highest concentration used (5×10^{-4} M), a shift in the excitation maxima from 300 nm to 327 nm (2753 cm^{-1}) was observed; in addition, the appearance of a peak at 276 nm was observed. A comparison of the spectra is shown in Figure 4-7.

Table 4-5. Spectral Characteristics of Several Molecules Under Various Conditions

Compound/Conc	Conditions	EX	EM	Comments
Fluorene (2×10^{-5} M)	β -CD/DBE	271, <u>300</u> ^a	370, <u>429</u> ^a , 465, 479	weak signals
	β -CD/BE	<u>300</u> ^a	<u>414</u> ^a , 462, 473, 477, 487	weak signals
	β -CD/BP	283(sh), <u>302</u> ^a	<u>421</u> ^a , 464, 473, 479(sh), 488(sh)	weak signals; BP most effective in inducing phosphorescence
Fluoranthene (2×10^{-5} M)	β -CD/DBE	<u>281</u> ^a , 347	436(sh), <u>474</u> ^a , 483(sh)	no phosphorescence observed
	β -CD/BE	282, 321(sh), <u>344</u> ^a	434, <u>466</u> ^a , 476	no phosphorescence observed; peak better resolved
	β -CD/BP	<u>279</u> ^a , 349	435, <u>472</u> ^a	peaks better resolved
Fluoranthene (4×10^{-5} M)	γ -CD/DBE	<u>352</u> ^a	437, 472, <u>546</u> ^a , 561(sh) 592	moderate signals peaks
	γ -CD/BP	281, <u>340</u>	431, <u>470</u>	no phosphorescence observed
	γ -CD/BP	<u>281</u> ^a , 350	438(sh), <u>475</u> ^a , 545, 591	not as well resolved; however peak at 545 nm more intense than w/DBE
1-Nitropyrene (2×10^{-5} M)	β -CD	271, <u>330</u> ^a	371, 382, <u>392</u> ^a , 411, 544, 617	moderate signals; phosphorescence observed w/out heavy atom
	β -CD/DBE	ND	372, 382, <u>392</u> ^a , 418, <u>544</u> ^a	moderate signals
	β -CD/BE	ND	371, 383, <u>391</u> ^a , 414(sh), 452, 533, 597	moderate signals; weak phosphorescence signals
1-Nitropyrene (2×10^{-5} M)	β -CD/BP	ND	371, 384, <u>389</u> ^a , 422(sh), 469, 546, 592	weak signals
	γ -CD	270, <u>334</u> ^a	371, 382, <u>389</u> ^a , 416, 542	weak signals
	γ -CD/DBE	N/D	372, 380, 385, 417, 466, <u>564</u> ^a	weak signals except at $\lambda = 564$ nm
				564 - excimer peak

Table 4-5. continued

Compound/Conc	Conditions	EX	EM	Comments
9-Nitroanthracene (2×10^{-5} M)	γ -CD/BE	N/D	317, 382, 390, 417 ^a , 547	weak signals
	γ -BP	N/D	372, 393, 418, 466, <u>573</u> ^a	lg. excimer peak; weak signals otherwise; 418 nm peak most intense in monomer fluorescence spectra
	β -CD	<u>256</u> ^a , 368	403, <u>423</u> ^a , 452, 465	moderate signals
	β -CD/DBE	<u>261</u> ^a , 365	406, <u>422</u> ^a	weak-moderate signals observed
γ -CD/BE	β -CD/BE	<u>258</u> ^a , 370	400, <u>424</u> ^a	moderate signals observed
	β -CD/BP	<u>265</u> ^a , 369	<u>423</u> ^a , 451, 467, 479	weak signals
	γ -CD	<u>262</u> ^a , 367	<u>420</u> ^a	weak signals
	γ -CD/BE	<u>260</u> ^a , 371	411(sh), <u>426</u> ^a , 453	moderate signals
2-Nitrofluorene (2×10^{-5} M)	γ -CD/BP	<u>251</u> ^a , 284, 305(sh)	403, <u>426</u> ^a , 464, 481(sh)	moderate signals
	β -CD	286	384	moderate signals
	β -CD/DBE	286	355, 399, <u>417</u> ^a , 466, 481, 490	weak-moderate signals
	β -CD/BE	N/D	<u>417</u>	weak signals
2-Nitrofluorene (2×10^{-5} M)	β -CD/BP	N/D	<u>416</u> ^a , 452, 465, 481, 489	very weak signals
	γ -CD/DBE	282	<u>417</u> ^a , 462, 481, 493	very weak signals
	γ -CD/BE	N/D	<u>417</u> ^a	very weak signals
	γ -CD/BP	N/D	<u>420</u> ^a , 449, 466, 481, 490	moderate signals

a: underlined peaks most intense peak.

Table 4-6. Analytical Figures of Merit for Compounds Included in CDs

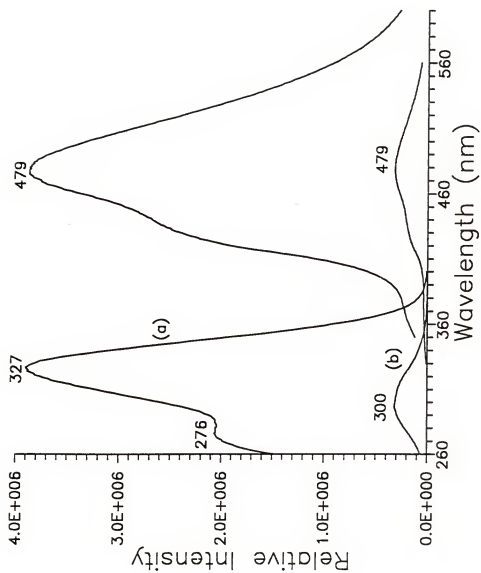
Compound	$\lambda_{ex}/\lambda_{em}$ (nm)	Conditions	UAR ^a (M)	Slope ^b	Correlation Coefficient	LOD ^c (M)
Acenaphthene	290/515	β -CD/BE.	5 E-7 - 1 E-4	0.94	0.997	6 E-8
7,8-Benzoflavone	285/429	γ -CD/BP	5 E-9 - 1 E-6	0.85	0.999	2 E-9
1-Naphthol	300/479	β -CD/BP	1 E-8 - 1 E-4	0.94	0.998	8 E-9

a: Useful analytical ranges, corresponding to the ratio of the upper concentration of linearity (within 5%) and the limit of detection.

b: Slope calculated from the log-log point.

c: Limit of detection, defined as the concentration of the analyte giving a signal-to-noise ratio of 3.

Fig. 4-7. Comparison of the luminescence spectra of 1- naphthol (a) 5×10^{-4} M
(b) 5×10^{-6} M.



CHAPTER 5

CONCLUSIONS

The previous studies using various types of organized media on solid substrate and in fluid solution have been shown to influence many luminescence properties of various molecules, and in many cases enhance these properties. First, from the paper substrate room temperature studies, one can conclude that surfactants of various ionic natures affect various classes of compounds in various ways. For the nonpolar PAHs, the phosphorescence intensities were enhanced by anionic surfactants, whereas with nonionic and cationic surfactants, quenching of the phosphorescence signals was observed.

For the phenothiazine derivatives, although there were many certain similarities in the behavior of the various types of surfactants, for example, the greatest intensities and enhancements were observed in the presence of anionic surfactants; there were also other differences. In the presence of nonionic and cationic surfactants (in the presence and absence of thallium) enhancement of the signals rather than quenching was observed. Although I⁻ was not used as a heavy atom in these studies, it is thought that in combination with these surfactants (especially the cationic surfactants), that the enhancement in signals observed for the PAHs would have been similar, or even better in the case of the phenothiazine derivatives.

Secondly, especially in the case of the PAHs, the surfactants afforded some protection from photochemical degradation of the analyte,

compared to the cases where the analytes were spotted from hexane solutions. Thirdly, limits of detection for PAHs and phenothiazine derivatives were better by factors of 2-10. Absolute limits of detection for PAHs ranged from 0.2 - 3 ng in the presence of surfactant, compared to 0.8 ng - 20 ng in its absence. Similar LODs were obtained for the phenothiazine derivatives.

Although the study of magnetite particles encapsulated in DHP vesicles was not as analytically useful as hoped, the luminescence properties of various molecules were affected in the presence of magnetite. In the future, if there is a way to increase the amount of magnetite in the vesicles, more dramatic effects on these luminescence properties may be observed.

Alcohols (brominated and their nonbrominated counterparts) were seen to affect the luminescence properties of molecules of various sizes in CDs with various internal cavity sizes. The extent to which various luminescence properties were affected seemed to be related to the stability of the ternary complex formed. There seems to be an intimate relationship between length of alcohol, size and shape of molecule and size of CD used. The limits of detection for the three molecules in this study were in the nanomolar range.

Cyclodextrins provide a selectivity based on lumiphor size and shape that the other types of organized media do not provide. This selectivity can also be viewed in terms of its ability to effectively perform an in situ separation spectroscopically by selectively complexing the desired species and repelling interferents (135). Future studies might investigate the combination of longer and/or bulkier alcohols (brominated and iodated) with molecules of various sizes and shapes in order to

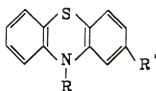
establish the right combination of alcohol-lumiphor-CD complex, where even better analytical figures of merit could be obtained.

APPENDIX A

The Structures of Several Phenothiazine Derivatives

Appendix A illustrates the structures of the eleven phenothiazine derivatives used in the study of paper substrate room temperature phosphorescence of phenothiazine derivatives enhanced by thallium (I) nitrate and surface-active agents. For a full discussion, refer to chapter 2.

Fig. A-1. The structures of the eleven phenothiazine derivatives used in this study.



Name	R	R'
Chlorpromazine HCl	$(\text{CH}_2)_3 \text{N}(\text{CH}_3)_2$	Cl
Fluphenazine HCl	$(\text{CH}_2)_3 \text{N} \langle \text{ring} \rangle \text{NCH}_2\text{CH}_2\text{OH}$	CF_3
Perphenazine	$(\text{CH}_2)_3 \text{N} \langle \text{ring} \rangle \text{NCH}_2\text{CH}_2\text{OH}$	Cl
Promazine HCl	$(\text{CH}_2)_3 \text{N}(\text{CH}_3)_2$	H
Thiopropazate HCl	$(\text{CH}_2)_3 \text{N} \langle \text{ring} \rangle \text{NCH}_2\text{CH}_2\text{OOCCH}_3$	Cl
Thioridazine HCl	$\text{CH}_2\text{CH}_2 \langle \text{ring} \rangle \text{N} \begin{array}{c} \text{CH}_3 \\ \end{array}$	3CH_3
Trifluoperazine (HCl) ₃	$(\text{CH}_2)_3 \text{N} \langle \text{ring} \rangle \text{NCH}_3$	CF_3
Trifluopromazine HCl	$(\text{CH}_2)_4 \text{N}(\text{CH}_3)_2$	CF_3
Trimiprazine tartarate	$\text{CH}_2 \text{CH} \begin{array}{c} \text{CH}_2\text{C}(\text{CH}_3)_2 \\ \\ \text{CH}_3 \end{array}$	H
Trichloroperazine adicylate	$(\text{CH}_2)_3 \text{N} \langle \text{ring} \rangle \text{NCH}_3$	Cl
Serentil	$\text{CH}_2 \text{CH}_2 \langle \text{ring} \rangle \text{N} \begin{array}{c} \text{CH}_3 \\ \end{array}$	CH_3SO

APPENDIX B

Spectra of Acenaphthene, Pyrene, and Phenanthrene Under Various Experimental Conditions

Appendix B shows the effect of β -CD and γ -CD with various alcohols (brominated and nonbrominated) on the luminescence spectra of three compounds--acenaphthene, pyrene and phenanthrene.

Fig. B-1. Acenaphthene (1×10^{-4} M) in water.

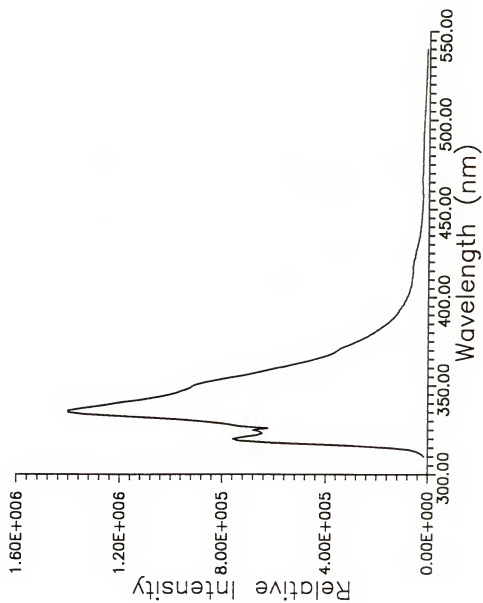


Fig. B-2. Acenaphthene (1×10^{-4} M) in β -CD (1×10^{-3} M).

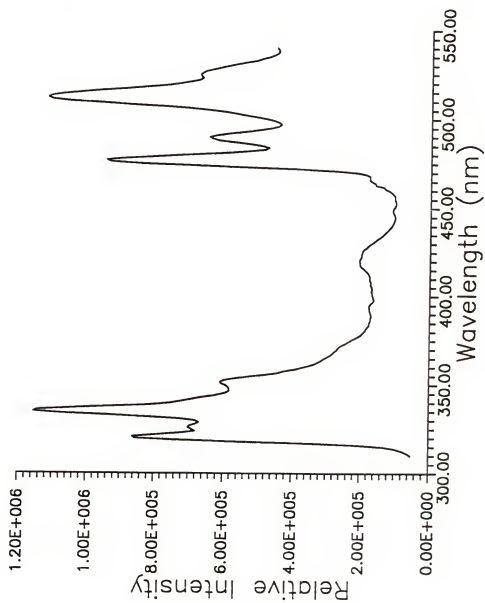


Fig. B-3. Acenaphthene (1×10^{-4} M) in β -CD (1×10^{-3} M) with BE (1.4×10^{-2} M).

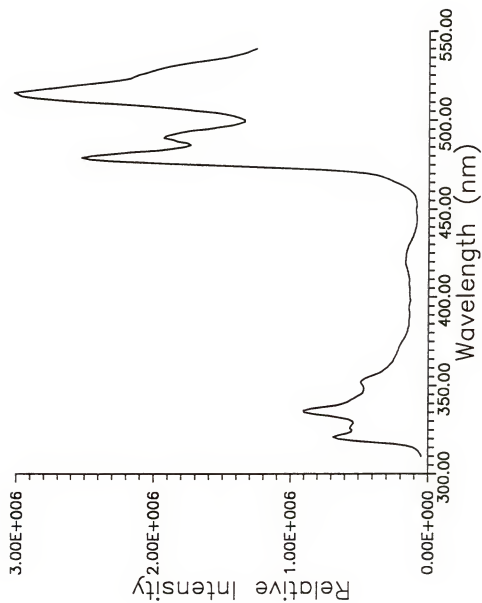


Fig. B-4. Acenaphthene (1×10^{-4} M) in γ -CD (1×10^{-3} M).

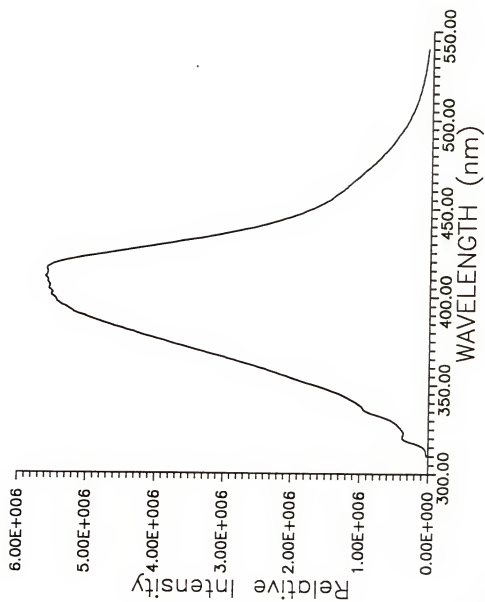


Fig. B-5. Acenaphthene (1×10^{-4} M) in γ -CD (1×10^{-3} M) with ethanol (1.4×10^{-2} M).

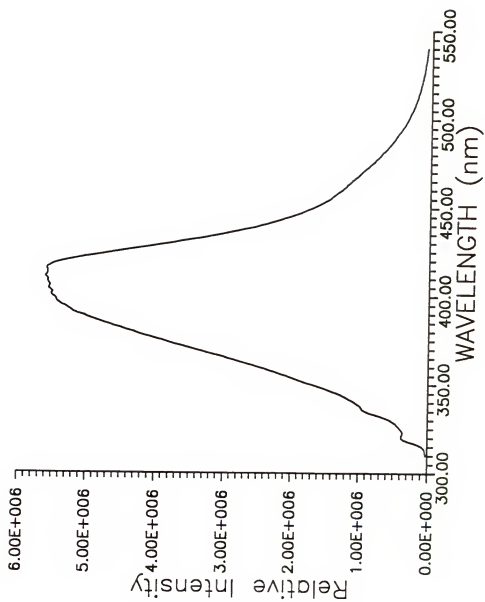


Fig. B-6. Acenaphthene (1×10^{-4} M) in γ -CD (1×10^{-3} M) with BP (1.4×10^{-2} M).

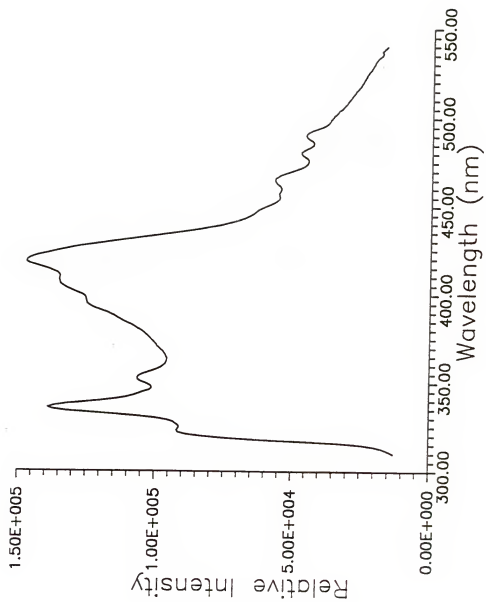


Fig. B-7. Acenaphthene (2×10^{-6} M) in γ -CD (1×10^{-3} M) with BP (1.4×10^{-2} M).

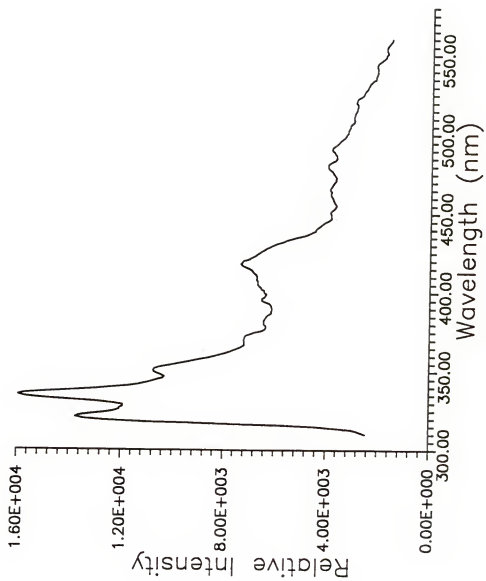


Fig. B-8. Acenaphthene (2×10^{-6} M) in γ -CD (1×10^{-3} M) with DBE (1.4×10^{-2} M).

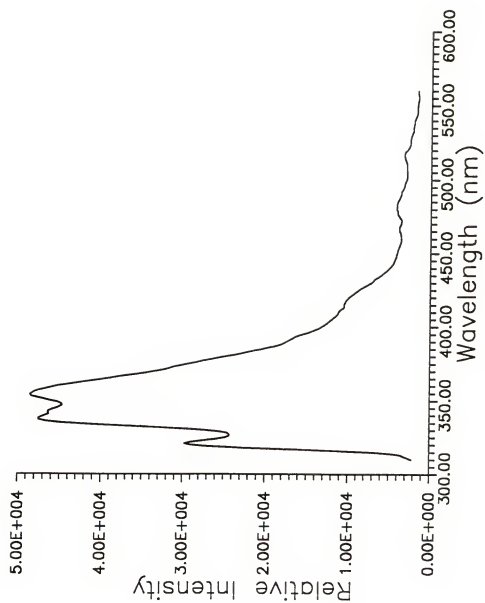


Fig. B-9. Pyrene (1×10^{-4} M) in water.

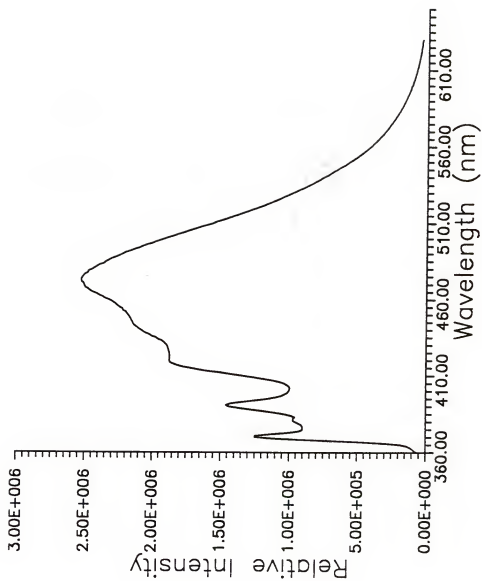


Fig. B-10. Pyrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with ethanol (1.4×10^{-2} M).

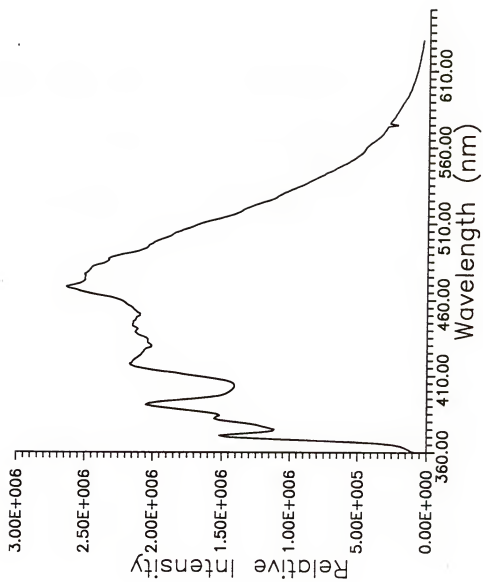


Fig. B-11. Pyrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with 2-propanol (1.4×10^{-2} M).

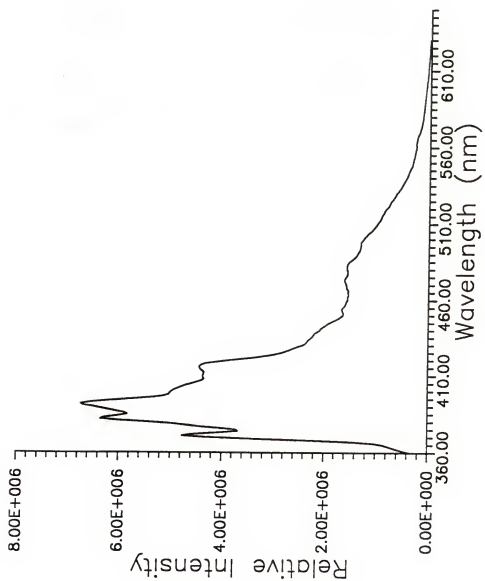


Fig. B-12. Pyrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with BE (1.4×10^{-2} M).

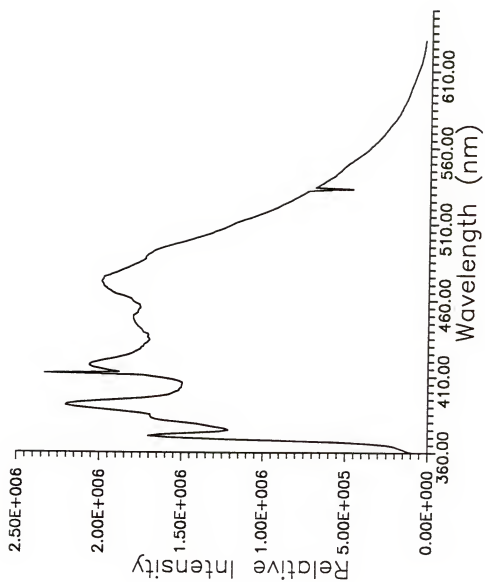


Fig. B-13. Pyrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with BP (1.4×10^{-2} M).

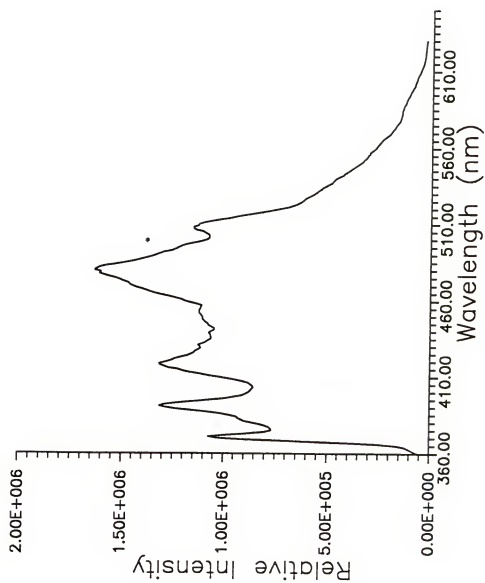


Fig. B-14. Pyrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with DBE (1.4×10^{-2} M).

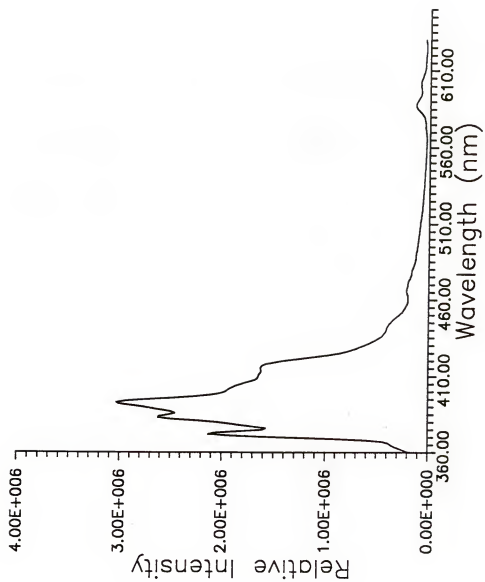


Fig. B-15. Pyrene (1×10^{-4} M) in γ -CD (1×10^{-3} M).

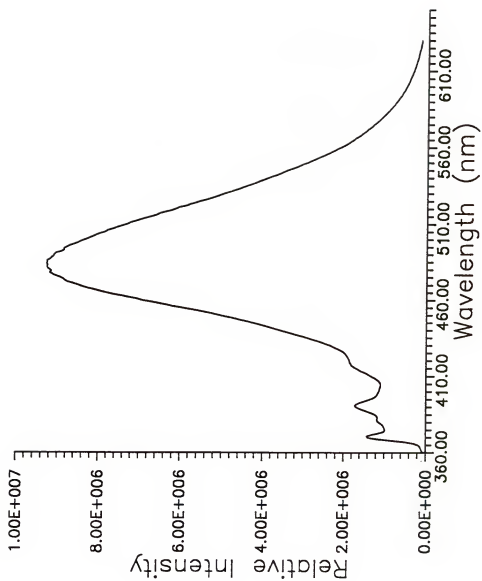


Fig. B-16. Pyrene (1×10^{-4} M) in γ -CD (1×10^{-3} M) with ethanol (1.4×10^{-2} M).

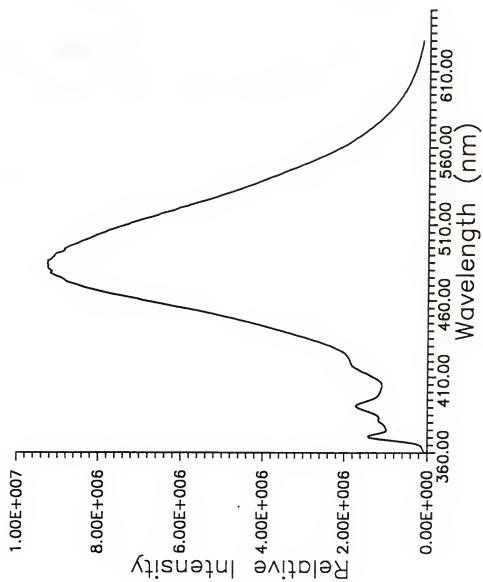


Fig. B-17. Pyrene (2×10^{-6} M) in γ -CD (1×10^{-3} M).

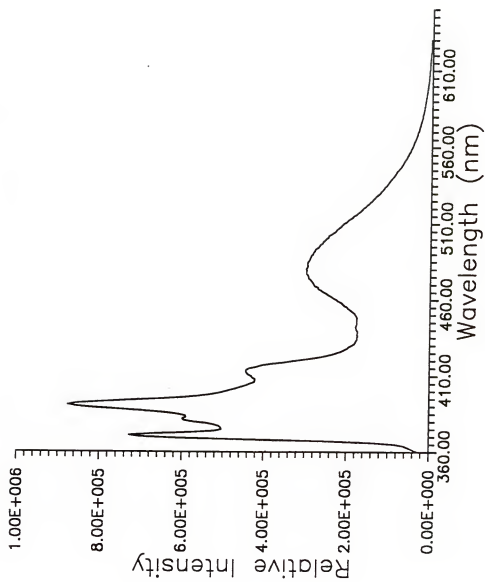


Fig. B-18. Phenanthrene (1×10^{-4} M) in water.

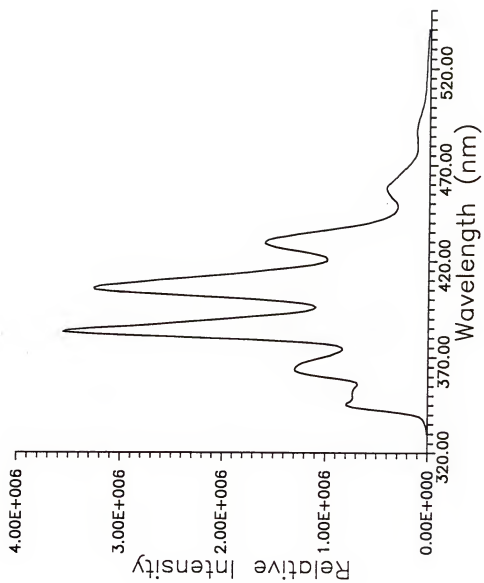


Fig. B-19. Phenanthrene (1×10^{-4} M) in β -CD (1×10^{-3} M).

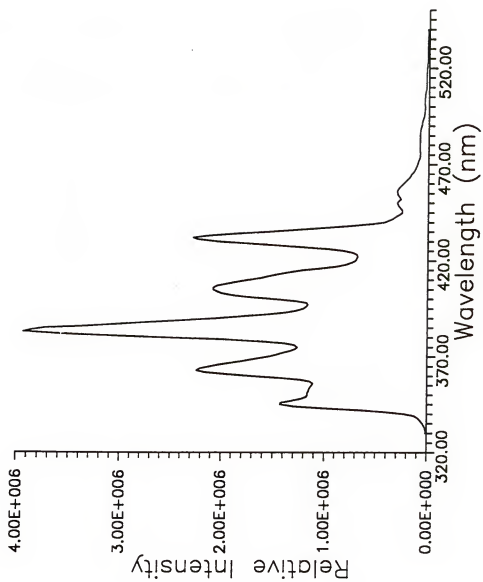


Fig. B-20. Phenanthrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with ethanol (1.4×10^{-2} M).

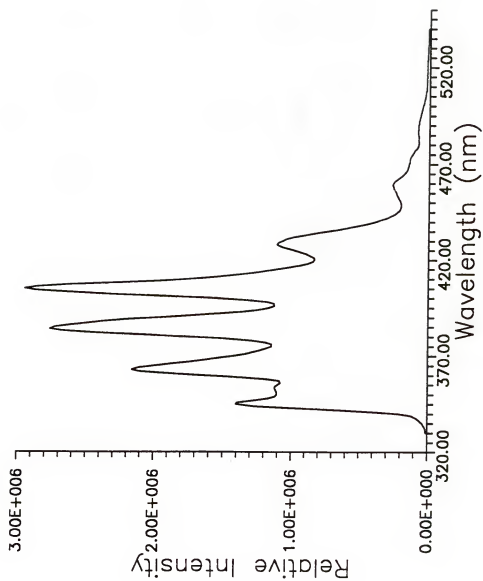


Fig. B-21. Phenanthrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with 2-propanol (1.4×10^{-2} M).

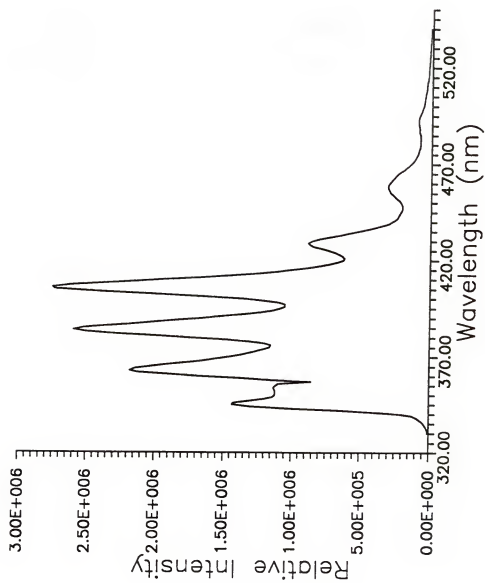


Fig. B-22. Phenanthrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with BE (1.4×10^{-2} M).

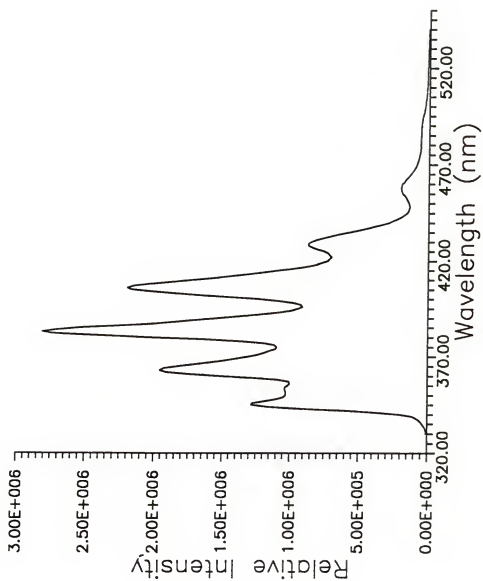


Fig. B-23. Phenanthrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with BP (1.4×10^{-2} M).

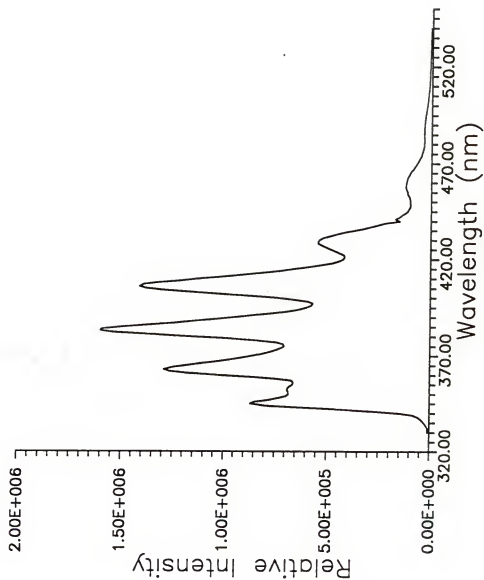


Fig. B-24. Phenanthrene (1×10^{-4} M) in β -CD (1×10^{-3} M) with DBE (1.4×10^{-2} M).

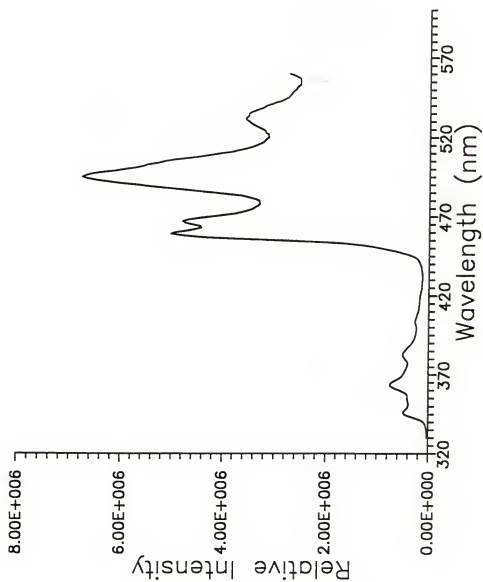


Fig. B-25. Phenanthrene (1×10^{-4} M) in γ -CD (1×10^{-3} M).

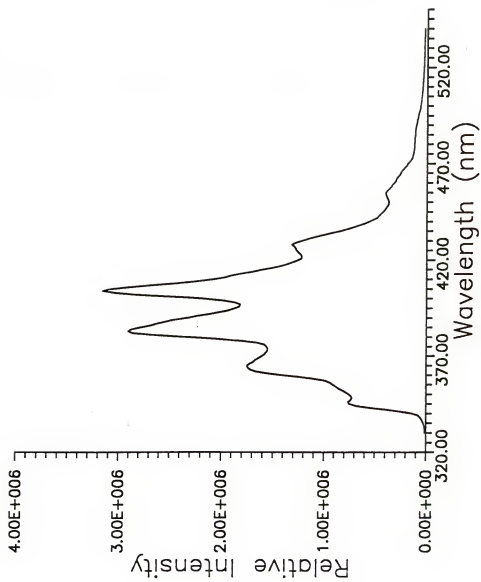


Fig. B-26. Phenanthrene (1×10^{-4} M) in γ -CD (1×10^{-3} M) with ethanol (1.4×10^{-2} M).

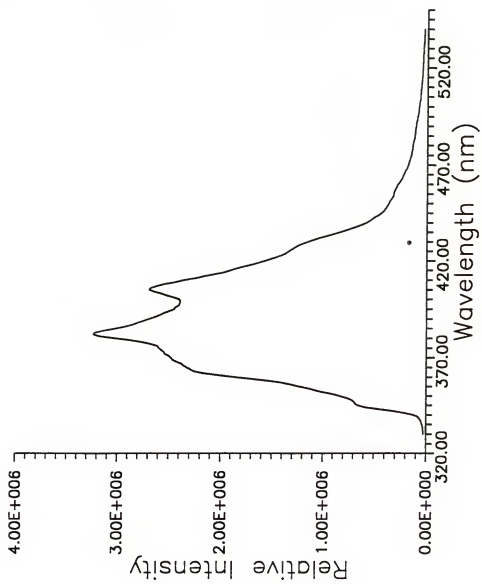


Fig. B-27. Phenanthrene (1×10^{-4} M) in γ -CD (1×10^{-3} M) with 2-propanol (1.4×10^{-2} M).

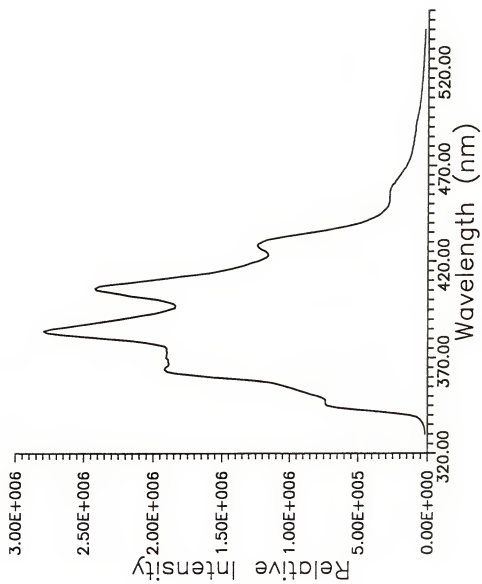


Fig. B-28. Phenanthrene (1×10^{-4} M) in γ -CD (1×10^{-3} M) with BE (1.4×10^{-2} M).

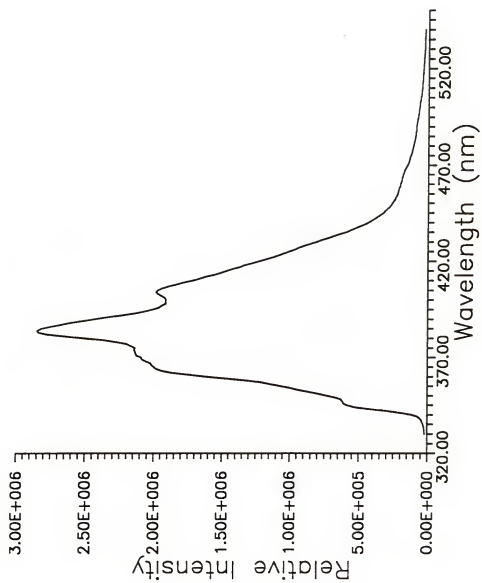


Fig. B-29. Phenanthrene (1×10^{-4} M) in γ -CD (1×10^{-3} M) with BP (1.4×10^{-2} M).

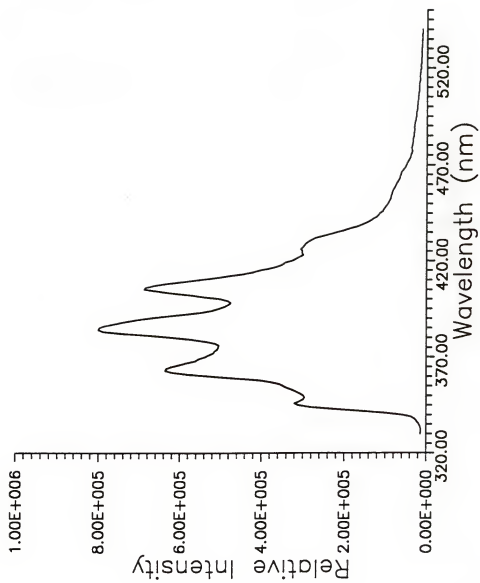
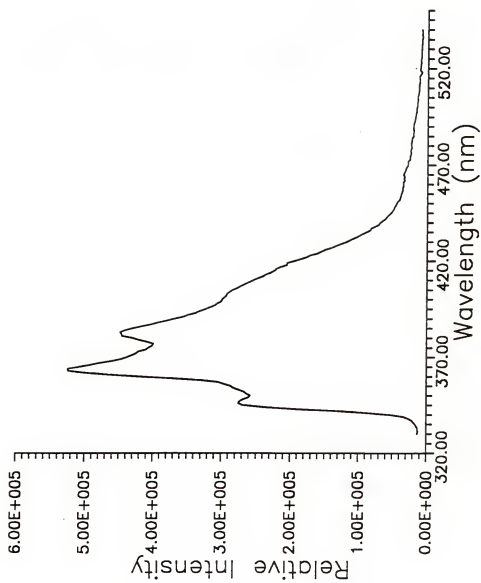


Fig. B-30. Phenanthrene (1×10^{-4} M) in γ -CD (1×10^{-3} M) with DBE (1.4×10^{-2} M).



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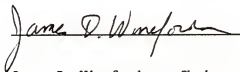
BIOGRAPHICAL SKETCH

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